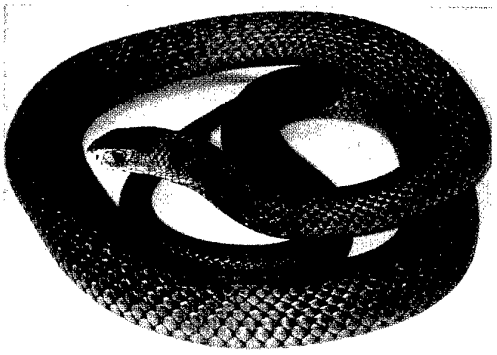
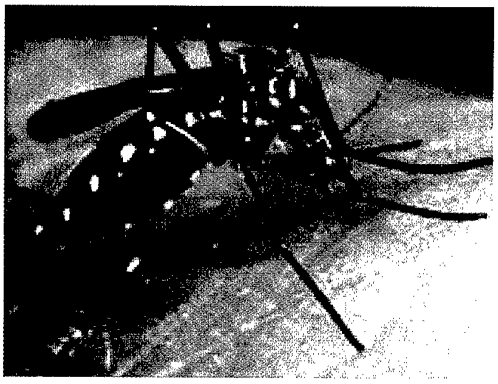
6th ASIA-PACIFIC CONGRESS ON ANIMAL, PLANT AND MICROBIAL TOXINS
&
11th ANNUAL SCIENTIFIC MEETING OF THE AUSTRALASIAN COLLEGE OF TROPICAL MEDICINE

Cairns, Australia

July 8-12, 2002





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Cover photos were provided by Wayne Hodgson (spider), Peter Mirtschin (snake), and Bruce Livett & David Paul (cone shell)

Title: 6th Asia Pacific Congress on Animal, Plant and Microbial Toxins
and 11th Annual Scientific Meeting of the
Australasian College of Tropical Medicine
Published in 2002 by International Society for Toxinology and Australasian College of Tropical Medicine

ISBN 0-9581116-0-X

Printed in Australia

Welcome

On behalf of the International Society for Toxinology and the Australasian College of Tropical Medicine, we have great pleasure in welcoming you to this, the 6th ASIA-PACIFIC CONGRESS ON ANIMAL, PLANT AND MICROBIAL TOXINS and the 11th ANNUAL SCIENTIFIC MEETING OF THE AUSTRALASIAN COLLEGE OF TROPICAL MEDICINE, at the Cairns Colonial Club Resort, Cairns, Australia.

This conference brings together experts in fields ranging from molecular toxinology to clinical studies of envenomation to tropical medicine. It comes at a time of many exciting developments in our respective fields. Our understanding of how toxins function is being enhanced by the tools developed for the rapidly growing fields of genomics and proteomics, while structural biology is making major advances in defining the structures of both toxins and their molecular targets. The same holds true for the toxins and antigens underlying viral, bacterial and parasitic diseases. The Northern Australian locality for this meeting proved very appropriate considering recent tragic events surrounding Irukandji envenomation. It also emphasizes the potency of our uniquely Australian venomous fauna and their direct and continuing role in human health. Clearly much remains to be learned about the composition and mode of action of many venoms, as well as the biology of venomous species. And while the world has come to fear toxins associated with bio-terrorism, concurrently it is embracing other toxins as therapeutics.

The intersections of toxinology and tropical health are many and varied. We thought it was particularly timely to address the challenges of emerging and re-emerging infectious diseases and biodefence. In addition, given the length of time since the last IST meeting in Australia, we also thought it appropriate to reflect on the achievements of some of those dedicated toxinologists and tropical medical doctors on whose shoulders we stand. As history shows, there continues to remain many interesting and challenging questions to answer in the fields of toxinology and tropical medicine, and we are sure that many of these will be discussed at our conference. The scientific program promises to be an exciting one, and we trust that a mix of stimulating science and good social interaction will make this meeting a worthwhile and enjoyable experience for everyone.

The local Organising Committee has done an excellent job and we would like to take this opportunity to thank them for their splendid effort. Only those of you who have been directly involved in organising a meeting such as this will appreciate how much work is required. Thanks also to our Scientific Programme Committee for their efforts in putting together an exciting program of talks and posters. If you have any questions or need any assistance during the meeting, please contact one of us.

Finally, we would also like to thank our sponsors, who are listed on the following page. Their generous support is greatly appreciated and their participation provides us with a valuable opportunity to learn of the latest developments in the tools of our trade.

We are delighted to welcome you to Cairns and hope you will enjoy the meeting.

Ray Norton, Chair
Ken Winkel, ACTM Scientific Program Convenor
On behalf of the Organising Committee

Sponsors

The following companies have provided financial support for the conference. We thank them for their generous contributions.

Major Sponsors

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Elsevier Science BV

Great Barrier Reef Research Foundation

Oxford University Press

Spider Pharm Inc

Venom Supplies Pty Ltd

General Information

Registration

The conference registration desk will operate in the foyer of the Cairns Colonial Club Resort Conference Centre from 2-4:30 p.m. on Sunday and 8-10 am on Monday.

Delegates arriving at other times should check in at the hotel registration desk then obtain their registration materials from Wayne Hodgson at the conference registration desk and settle any outstanding balances at that time. After Monday morning, the registration desk will be staffed only during breaks in the scientific programme.

Accommodation

Delegates who have booked accommodation through the conference will be housed at the Cairns Colonial Club Resort.

The balance of your accommodation and breakfast package, plus any extras such as bar accounts, meals and phone calls, must be settled with the hotel registration desk prior to departure. Check-out time is 10 am. Luggage can be stored in the Lobby area of the Hotel.

Lectures

All lectures will be held in the Cairns Colonial Club Resort Conference Centre. Most sessions will be held in the Lockhart Room (large lecture hall). When concurrent sessions are scheduled, both the Lockhart Room and the Jardine Room (first floor of Conference Centre) will be used.

Lectures will be of 45 minutes duration, including 5 minutes discussion, or 25 minutes, including 5 minutes discussion, or 15 minutes, including 3 minutes discussion. Please check the programme for your allocated time.

A slide preview room equipped with a slide projector and a PC laptop will be available throughout the conference. For those with computer-based presentations, please load your presentation onto the conference PC laptop well before the start of your session. This will be running Powerpoint 2000. Speakers should save their talks as 2000 or an earlier version (eg. 97). Please do not use Powerpoint XP. The conference laptops will accept 3.5" disks or CDs. For those who wish to use their own PC or Mac during their talk, please arrange this with the AV staff well before the start of your session.

Speakers using slides are asked to load the slides into a carousel, check them, then give the loaded carousel to the projectionist at least 30 minutes before the start of their session.

Overhead projectors will be available for all sessions.

Posters

There will be two poster sessions, one on Monday afternoon and one on Thursday afternoon. Posters in Session 1 can be mounted on Sunday from 2 pm and should be removed by 6 pm on Tuesday. Posters in Session 2 can be mounted from 8 am Wednesday and should be removed by 12 noon on Friday.

All posters will be displayed in the Cairns Colonial Club Resort Conference Centre adjacent to the Lockhart Room, which is also the venue for the trade display and morning and afternoon coffee. The space allowed for posters is 1.0 metre by 1.0 metre. Posters will be fixed to the display boards using Velcro, which will be provided in the poster room.

Messages

Messages for delegates and other notices can be displayed on a Messages board near the entrance to the Cairns Colonial Club Resort Conference Centre (Phone: +61 7 4053 8805 Fax: +61 7 4032 1182).

Tea Breaks

Tea and coffee will be provided during the scheduled breaks in the Cairns Colonial Club Resort Conference Centre. There will be no tea or coffee on Wednesday or Friday afternoons.

Meals

For registrants organising their accommodation through the conference, a tropical breakfast is included. A full breakfast may be purchased if required.

The Informal Drinks on Sunday night are open to all registrants and their partners. The Welcoming Reception on Monday night and the Conference Dinner on Thursday night are included in the registration fee. For non-registrants, tickets for the functions on Monday and Thursday nights should be purchased in advance or at registration.

All of these functions will be held at the Cairns Colonial Club Resort.

Trade Display

The trade display will be held in the Cairns Colonial Club Resort Conference Centre adjacent to the Lockhart Room.

Company representatives will be on hand during morning and afternoon tea breaks, at the Welcoming Reception on Monday night, and during the Poster Sessions on Monday and Thursday afternoons. Please take advantage of the opportunity to discuss their latest offerings with the company representatives at these times.

Transport

Cairns Colonial Club Resort is situated only 7 kilometres (4.5 miles) from Cairns International Airport, and 6 kilometres (4 miles) from the Cairns City Centre.

The resort offers complimentary transfers to the resort from all major arrival points. A taxi from Cairns airport to Cairns Colonial Club Resort will costs around A$10. Similar taxi fares apply for the bus station and railway station in Cairns

A courtesy cityshuttle runs every hour throughout the day between 8 am and 6 pm. The coach departs the resort on the hour and drops off and picks up at both the City Centre (10 min past the hour) and Cairns Central Shopping Centre (15 min past the hour).

Tours and Activities

The conference will include a number of social activities centred around the natural beauty of the Great Barrier Reef and the tropical rainforest, and Australia's unique cultural heritage. A choice of two excursions is available on Wednesday afternoon:

A boat trip to nearby Green Island resort, where you can swim, snorkel, bushwalk or just relax.

Or a cable car ride through the lush tropical rainforest to the elevated village of Kuranda, with its famous market.

The Wednesday excursion is included in the registration fee for conference participants. Additional tickets may be purchased for accompanying persons.

A further optional excursion (at additional cost) is available to registrants and accompanying persons to the Tjapukai Aboriginal Cultural Park on Tuesday afternoon. Enjoy lunch and learn about the fascinating life and culture of Australia's indigenous people.

Bus transport and lunch will be provided for all excursions. Casual dress is appropriate, and hats, sunscreen and comfortable shoes are suggested for outdoor activities. Bring a swimsuit and towel for the Green Island excursion.

There is a Tour Desk on site, whose staff can give advice on a range of tours and activities to suit your needs and budget, as well as organising any bookings for you. Car and bike hire can also be arranged.

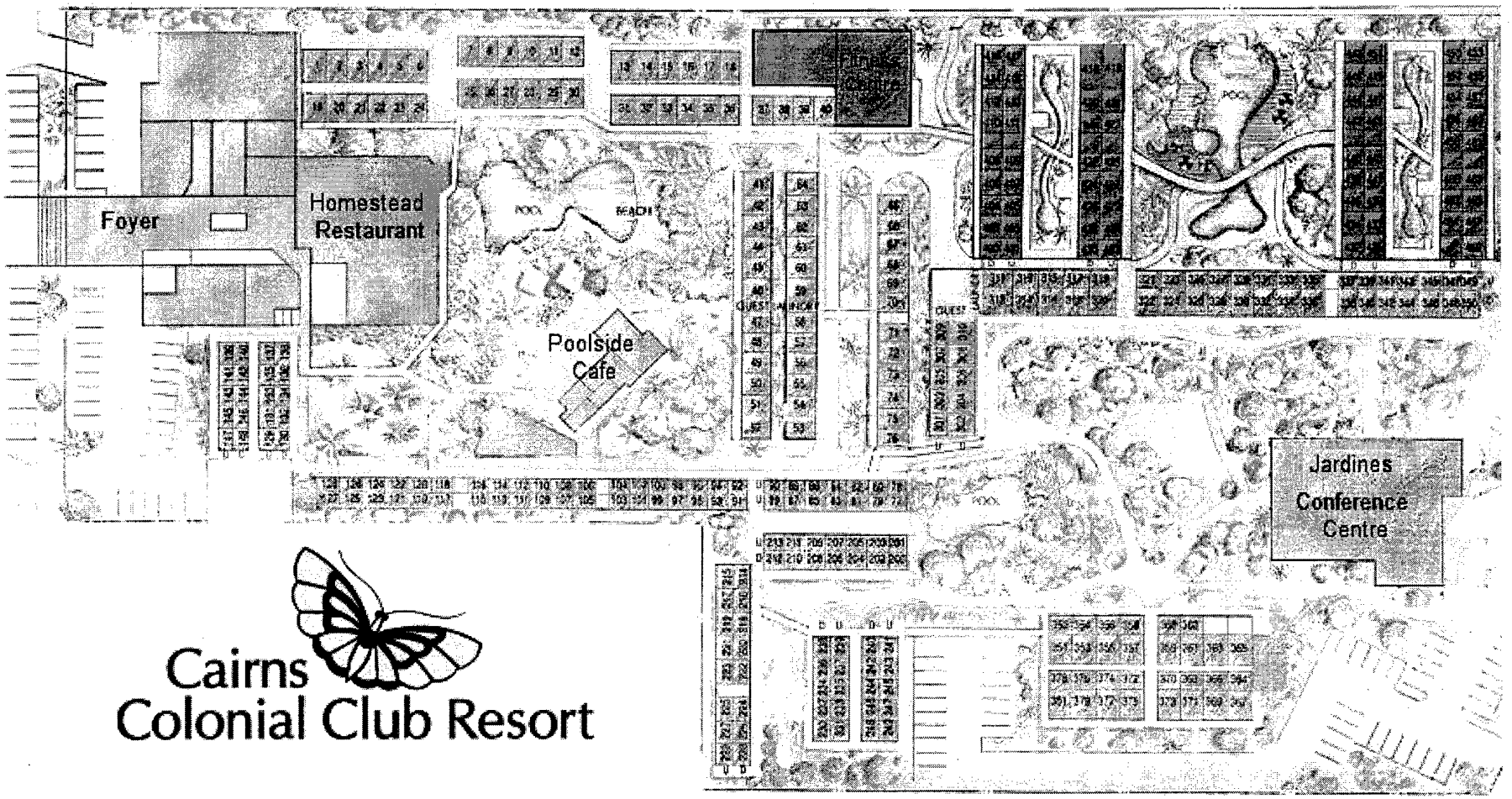
Weather

July is in the middle of the tropical dry season (May to October). Average daily temperatures are around 25-27°C, dropping to 17-20°C at night. Light, casual clothing is recommended. A sweater or jacket may be required for evenings.

Dress

Smart casual dress is suggested for the conference dinner on Thursday evening. Informal dress is appropriate for all other conference functions, including welcoming drinks on Sunday evening and the informal mixer on Monday evening.

We trust you will enjoy the conference.


Foyer
Homestead
Restaurant
Poolside
Cafe
Jardines
Conference
Centre
Cairns
Colonial Club Resort

Program Summary (*Please check times against full program*)

	SUNDAY, 7 July	MONDAY, 8 July	TUESDAY, 9 July	WEDNESDAY, 10 July	THURSDAY, 11 July	FRIDAY, 12 July
08:00-08:45			ACTM Business Meeting			
08:45-09:00		Conference Opening				Symposium 18
09:00-11:00		Symposium 4 Proteonomics, Genomics and Toxin Structure & Function	Symposium 10 Clinical Toxinology I	Symposium 14 Microbial and Malarial Toxins	Symposium 15 Applied Toxinology	Conotoxins
11:00-11:25		Morning Tea	Morning Tea	Morning Tea	Morning Tea	Morning Tea
11:25-13:00		Symposium 5 Snake Toxins Symposium 6 Tropical Diseases: Molecular	Symposium 11 Clinical Toxinology II	Excursions: Green Island or Skyrail/Kuranda (11:25-17:30)	Symposium 16 Arachnid Toxins I	Symposium 19 Marine Toxins I
13:00-14:00		Lunch	Lunch		Lunch	Lunch
13:00-15:30			Tjapukai Visit (optional) Workshop 1 Cubozoan Workshop			
14:00-14:15		Conference Photograph				
14:00-14:30					Symposium 17 Arachnid Toxins II	
14:00-15:30	Symposium 1 Travel Medicine I		Workshop 2 Emerging and Re-Emerging ID			Symposium 20 Marine Toxins II
14:15-15:30		Symposium 7 Snake Toxins II Symposium 8 Tropical Diseases: Clinical				
14:30-15:30					IST Business Meeting	
15:30-16:00	Afternoon Tea	Afternoon Tea	Afternoon Tea		Afternoon Tea	Concluding Remarks
16:00-17:00	Symposium 2 Travel Medicine II					
16:00-17:30		Poster Session 1	ACTM Convocation Symposium 12 Snake Toxins III		Poster Session 2	
17:00-17:30	Symposium 3					
17:30-19:30	Informal Drinks					
18:00-20:00		Light Dinner/Mixer				
19:30-21:00			Symposium 13 Clinical Toxinology III			
19:30-23:00					Conference Dinner	
20:00-21:00		Symposium 9 Tropical Diseases in Practice		Workshop 3 Antivenom Production		

SUNDAY, 7 JULY 2002

SYMPOSIUM 1 **14:00-15:30**

TRAVEL MEDICINE I

Chair: Peter Leggatt
Rural Training Unit, Cairns Base Hospital

AFTERNOON TEA **15:30-16:00**

SYMPOSIUM 2 **16:00-17:00**

TRAVEL MEDICINE II

Chair: John Heydon

Paper 1 **16:00** Healthcare for the polar tourist
Tingay, D.

Paper 2 **16:15** Travel medicine - the view from Everest
Heydon, J.

Paper 3 **16:30** Health problems in Bougainville - paradise lost?
Poprawski, D.

Paper 4 **16:45** Targeting vector behaviour and characteristics for effective malaria control
Durrheim, D.N., Govere, J.M.

SYMPOSIUM 3 **17:00-17:30**

TRAVEL MEDICINE III

Chair: John Heydon

Paper 1 **17:00** From snake bite to summit: the 2001 South Australian Everest expedition
Tingay, D., Winkel, K.

INFORMAL DRINKS **17:30-19:30**

MONDAY, 8 JULY 2002

CONFERENCE OPENING 08:45-09:00

SYMPOSIUM 4 09:00-11:00

PROTEOMICS, GENOMICS AND TOXIN STRUCTURE & FUNCTION

Chair: Dietrich Mebs

Paper 1 09:00 Protein engineering and the evolution of a ricin vaccine
Smith, L.A., Roxas, M., Olson, R., Wannemacher, C., Millard, C., LeClaire, R.

Paper 2 09:45 Global gene expression profiling of human genome following exposure to toxins - emerging field of toxinogenomics
Gopalakrishnakone, P., Srinivasan, K.N., Zhong, S., Pachiappan, A., Anath, C., Qi, Z.

Paper 3 10:10 How do snake neurotoxins block nicotinic acetylcholine receptors?
Fruchart-Gaillard, C., Gilquin, B., Cahngeuz, J.-P., Servent, D., Menez, A.

Paper 4 10:35 Animal venom proteomics
Stocklin, R.

MORNING TEA 11:00-11:25

SYMPOSIUM 5 11:25-13:00

SNAKE TOXINS

Chair: Andre Menez

Paper 1 11:25 Prothrombin activators from Australian snake venoms
Kini, R.M., Rao, V.S., Joseph, J.

Paper 2 11:50 Investigation of the structure, dynamics and folding of snake venom cardiotoxins
Kumar, T.K.S., Yu, C.

Paper 3 12:15 A peptide derived from the phospholipase A_2 inhibitor from *Python reticulatus* (PIP) effectively protects kainate-induced exocitotoxic neuronal injury in rat hippocampal slices
Thwin, M.-M., Ong, W.-Y., Fong, C.-W., Sato, K., Kodama, K., Gopalakrishnakone, P.

Paper 4 12:30 Snake α-neurotoxin binding site on the Egyptian cobra (*Naja haje*) acetylcholine receptor is masked by glycosylation
Takacs, Z., Wilhelmsen, K.C., Sorota, S.

Paper 5 12:45 Structure/functional study of jararhagin using recombinant fragments and monoclonal antibodies
Butera, D., Tanjoni, I., Gutierrez, J.M., Colombini, M., Fernandes, I., Moura-da-Silva, A.M.

MONDAY, 8 JULY 2002

SYMPOSIUM 6 **11:25-13:00**

TROPICAL DISEASES: MOLECULAR

Chair: Robert Hirst

Paper 1 **11:25** Molecular epidemiology of melioidosis in Australia
Currie, B.J.

Paper 2 **11:50** Catching a tiger by the tail: *in vitro* identification of potential vaccine candidates for melioidosis
Inglis, T.J.J., Woods, D.E.

Paper 3 **12:15** G-CSF in the treatment of severe melioidosis; recent developments
Cheng, A., Stephens, D., Anstey, N., Fisher, D., Huffam, S., Currie, B.

Paper 4 **12:30** G-CSF immunotherapy for treatment of acute disseminated murine melioidosis
Powell, K., Ulett, G., Hirst, R., Norton, R.

Paper 5 **12:45** The efficacy of *Clostridium botulinum* type C and D neurotoxin subunit vaccines evaluated in mice
Woodward, L.A., Oguma, K., Hirst, R.G.

LUNCH **13:00-14:00**

CONFERENCE PHOTOGRAPH **14:00-14:15**

SYMPOSIUM 7 **14:15-15:30**

SNAKE TOXINS II

Chair: P. Gopalakrishnakone

Paper 1 **14:15** Pioneers in Australian toxinology: from Joseph Banks to Charles Kellaway
Winkel, K.D.

Paper 2 **14:30** Isolation and pharmacological characterisation of a myotoxic PLA_2 from a death adder venom
Wickramaratna, J.C., Fry, B.G., Aguilar, M.I., Kini, R.M., Hodgson, W.C.

Paper 3 **14:45** Purification and cloning of pseudechetoxin that targets cyclic nucleotide-gated ion channels
Yamazaki, Y., Brown, R.L., Morita, T.

Paper 4 **15:00** Purification and partial characterisation of hyaluronidase and fibrinogenase from pit viper (*Agkistrodon halys*) venom
Yau, Y.H., Tan, L.T., Teoh, K.Y., Gopalakrishnakone, P., Khoo, H.E.

Paper 5 **15:15** WAPRINS: a new family of proteins from snake venoms
Ying, W.H., Hock, S.T., Desai, M., Fry, B.G., Kini, R.M.

MONDAY, 8 JULY 2002

SYMPOSIUM 8 **14:15-15:30**

TROPICAL DISEASES: CLINICAL

Chair: Peter Legatt

Paper 1 **14:15** The knowledge of North Queensland island ferry passengers about Irukandji jellyfish
Harrison, S., Leggat, P., Durrheim, D., Fenner, P., Swinbourne, A.

Paper 2 **14:30** Imported parasitic skin infections in Tasmania
Goldsmid, J.M., Bettiol, S.

Paper 3 **14:45** Managing death adder bite with prolonged pressure bandaging
Oakley, J.

Paper 4 **15:00** The green pit viper bite - an Irish adventure
Poprawski, D.

Paper 5 **15:15** Recent fatal botulism in South Africa
Frean, J., Arntzen, L., van den Heever, J., Perovic, O.

AFTERNOON TEA **15:30-16:00**

POSTER SESSION 1 **16:00-17:30**

Chair: Bruce Livett and Graham Nicholson

Snake Toxins and Clinical

Poster 1-1 Cloning and expression of a synthetic gene for Ohanin: a novel protein from King Cobra venom
Pung, Y.F., Kumar, P.P., Kini, R.M.

Poster 1-2 Nucleotide sequences of neurotoxin genes isolated from *Bungarus candidus* share homology with bungarotoxin genes of *Bungarus multicinctus* but not *Bungarus fasciatus*
Suntrarachun, S., Tirawatnapong, T., Pakmanee, N., Noiphorm, J., Sitprija, V.

Poster 1-3 Analysis of the transcriptome from *Bothrops insularis* venom glands: identification of new proteins through the generation of expressed sequence tags (ESTs)
Junqueira de Azevedo, I.L.M., Ho, P.L.

Poster 1-4 Molecular cloning and expression of a functional snake venom vascular endothelial growth factor (svVEGF) from the *Bothrops insularis* pitviper. A new member of the VEGF family of proteins
Junqueira de Azevedo, I.L.M., Farsky, S.H.P., Ho, P.L.S.

Poster 1-5 cDNA cloning of precursor of bradykinin-potentiating peptides and C-type natriuretic peptide from Thai snake, *Calloselasma rhodostoma*
Murayama, N., Suntrarachun, S., Noiphrom, J., Khow, O., Chanhome, L., Saguchi, K., Ohi, H., Fujita, Y., Sitprija, V., Higuchi, S.

Poster 1-6 A new gene structure of disintegrin family: a subunit of dimeric disintegrin have short coding region
Okuda, D., Koike, H., Morita, T.

Poster 1-7 Isolation and partial characterization of an anti-platelet aggregation and antitumoral protein from *Bothrops jararacussu* snake venom
Cintra, A.C., Toyama, M.H., Marangoni, S., Nonizo, A., Giglio, J.R., Sampaio, S.V.

Poster 1-8 The cDNA sequence of the non-enzymatic subunit of Pseutarin C, a prothrombin activator from the brown snake (*Pseudonaja textilis*) shows structural similarity to blood coagulation factor V
Rao, V.S., Swarup, S., Kini, R.M.

Poster 1-9 Occurrence of a novel *O*-linked xylose-GlcNAc disaccharide in trocarin, a factor Xa homolog from snake venom
Joseph, J.S., Valiyaveettil, M., Gowda, D.C., Kini, R.M.

Poster 1-10 Purification and characterization of a fibrin(ogen)olytic kininogenase from the venom of *Trimeresurus jerdonni* snake
Wei, J.-F., Jin, Y., Lu, Q.-M., Wei, Q., Wang, W.-Y., Xiong, Y.-L.

Poster 1-11 Purification and characterization of jerdofibrase, a serine protease from the venom of *Trimeresurus jerdonii* snake
Xiong, Y.-L., Jin, Y., Lu, Q.-M., Wei, J.-F., Li, D.-S., Wang, W.-Y.

Poster 1-12 Biochemical characterization of jerdofibrase-2, an alpha-fibrinogenase with high molecular weight isolatd from the venom of *Trimeresurus jerdonii*
Jia, Y.-H., Jin, Y., Lu, Q.-M., Li, D.-S., Wang, W.-Y., Xiong, Y.-L.

Poster 1-13 Purification and cloning of a novel C-type lectin-like protein with platelet aggregation activity from *Trimeresurus mucrosquamatus* venom
Wei, Q., Lu, Q.-M., Jin, Y., Li, R., Wei, J.-F., Wang, W.-Y., Xiong, Y.-L.

Poster 1-14 Characterization and cloning of a novel phospholipase A_2 from the venom of *Trimeresurus jerdonii* snake
Lu, Q.-M., Jin, Y., Wei, J.-F., Li, D.-S., Zhu, S.-W., Wang, W.-Y., Xiong, Y.L.

Poster 1-15 Amino acid sequence and toxicological properties of a phospholipase A_2 enzyme from *Hoplocephalus stephensi* snake venom
Pek, E.S., Rajaseger, G., Lu, J., Yeo, J., Moochhala, S., Kini, R.M.

Poster 1-16 An inflammatory agent, venom phospholipase A_2, modulates the expression of lung aquaporins
Cher, C.D.N., Armugam, A., Jeyaseelan, K.

Poster 1-17 Venom nerve growth factors as a modulator of Aquaporins in the brain
Koh, D.C.-I., Nair, R., Armugam, A., Ong, W.Y., Jeyaseelan, K.

Poster 1-18 Structural and functional characterization of a phospholipase A_2 myotoxin inhibitor from *Bothrops moojeni* snake plasma
Soares, A.M., Giglio, J.R., Guerra-Sa, R., Franca, S.C., Arantes, E.C.

Poster 1-19 *Micrurus phyrrocryptus* ("Mboi-Chumbe-Guazu") venom produces myotoxicity in rats and mice
de Roodt, A.R., Gimeno, E., Litwin, S., Dokmetjian, J.Ch., Estevev, J., Dolab, J.A., Paniagua, J.

Poster 1-20 Cloning and characterization of novel snake venom proteins, which block smooth muscle contraction
Morita, T., Yamazaki, Y., Koike, H., Sugiyama, Y., Motoyoshi, K., Wada, T., Hishinuma, S., Mita, M.

Poster 1-21 Pharmacological characterization of contraction induced by blomhotin, a novel peptide from the venom of *Agkistrodon halys blomhoffii*, in rat fundus
Samejima, Y., Iwasaki, E., Yanoshita, R.

Poster 1-22 Blomhotin-related peptides increase vascular permeability through mast cell activation
Yanoshita, R., Doi, Y., Samejima, Y.

Poster 1-23 A 17-mer phospholipase A_2-inhibitory peptide neutralizes the toxicity of candoxin, a non-conventional (weak) toxin from *Bungarus candidus*
Thwin, M.-M., Nirthanan, S., Sato, K., Kodama, K., Gopalakrishnakone, P.

Poster 1-24 Putative functional determinants of candoxin-induced blockade of muscle ($\alpha\beta\gamma\delta$) and neuronal α7 nicotinic acetylcholine receptors
Nirthanan, S., Charpantier, E., Gopalakrishnakone, P., Gwee, M.C.E., Khoo, H.E., Bertrand, D., Kini, R.M.

Poster 1-25 Two species of cobras distributed in the mainland of China contain similar short-chain neurotxins
Wang, W.-Y., Wei, J.-F., Lu, Q.-M., Jin, Y., Zhou, X.-D., Li, R., Xiong, Y.L.

Poster 1-26 Avidin-biotin optical immunoassay for the detection of *Trimeresorus popeorum* venom
Le, V.D., Gopalakrishnakone, P., Khoo, H.E.

Poster 1-27 The intraperitoneal toxicity in mice of a range of Australian and exotic snake venoms
Almeida, A., Winkel, K., Mirtschin, P.J., Kuchel, T.R.

Poster 1-28 Influences on venom yield in Australian tigersnakes (*Notechis scutatus*) and brownsnakes (*Pseudonaja textilis*: elapidae, serpentes)
Mirtschin, P.J., Shine, R., Nias, T.J., Dunstan, N.L., Hough, B.J., Mirtschin, M.

Poster 1-29 Development of a global internet resource for clinical toxinology
White, J., Staples, A., Bates, D., Chipillo, M.

Poster 1-30 Antivenom use in Australia; reports of use 1994-2002
White, J., Chipillo, M., Hobbs, P.

Poster 1-31 Suspected snake-bite: a prospective study of presentations to an emergency department in tropical Australia
Isbister, G.K., Currie, B.J.

Poster 1-32 Five years of snake envenoming in Far North Queensland
Barrett, R.H., Little, M.

Poster 1-33 Analysis of intensive care unit admissions for treatment of serious snakebite at Port Moresby General Hospital
Williams, D.J., Kevau, I.H., Hiawalyer, G.W., Leggat, P.A., Muller, R.

Poster 1-34 Bites by spiders of the family Theraphosidae in humans and canines
Isbister, G.K., Seymour, J., Gray, M.R., Raven, R.

Poster 1-35 Serum scorpion venom level and clinical variables according to severity grade in patients treated with Alacramyn™
Reyes, S., Garcia, W., Batalla, A., Escandon, C., Cabral, J., Gonzalea, C., Cabral, J., Paniagua, J., Alagon, A.

Poster 1-36 Pharmacokinetics of antiscorpion horse F(ab)2 fragments Alacramyn™ in 6 healthy volunteers
Alagon, A., Chavez-Haro, A., Vazquez, H., Paniagua, J.

Poster 1-37 Financial implications of jellyfish envenoming in Tropical North Queensland
Mulcahy, R., Pereira, P., Cullen, P., Little, M., Seymour, J., Carrette, T.

Poster 1-38 The incidence of myocardial injury in hospital patients with Irukandji syndrome
Halkidis, L.

Poster 1-39 A prognostic scoring system for acute melioidosis
Cheng, A.C.S., Jacups, S.P., Currie, B.J.

Poster 1-40 Today's traveller and diving adventures - how to survive your day in practice
Poprawski, D.M.

Poster 1-41 Health advice given by general practitioners for travellers from Australia
Seelan, S.T., Leggat, P.A.

Poster 1-42 Resources utilized by general practitioners for advising travellers from Australia
Seelan, S.T., Leggat, P.A.

Poster 1-43 Trends in antimalarial drugs prescribed in New Zealand 1993-1998
Leggat, P.A., Heydon, J.L.

Poster 1-44 Trends in antimalarial drugs prescribed in Australia 1992-1998
Leggat, P.A., Speare, R.

Poster 1-45 The prevalence of low back pain and related disability in Australian adults
Walker, B.F.

LIGHT DINNER AND MIXER **18:00-20:00**

SYMPOSIUM 9 **20:00-21:00**

TROPICAL DISEASES IN PRACTICE

Chair: John Heydon

Paper 1 **20:00** Rural and tropical medicine - what's that?
Schreuder, G.

Paper 2 **20:15** Realtime molecular epidemiology
Inglis, T.J.J.

Paper 3 **20:30** Microbiology teaching - its importance in tropical and travel medicine and a consideration of problems associated with its teaching at the undergraduate level to medical students
Goldsmid, J.M.

TUESDAY, 9 JULY 2002

ACTM BUSINESS MEETING **08:00-09:00**

SYMPOSIUM 10 **09:00-11:00**

CLINICAL TOXINOLOGY I

Chair: Ken Winkel

Paper 1	**09:00**	Biological and chemical defence: the threat of biological and chemical weapons *Robertson, A.G.*
Paper 2	**09:45**	Clinical Toxinology; a global and Australian perspective *White, J.*
Paper 3	**10:10**	The production of *Calloselasma rhodostoma* (CR) antivenom from egg yolk of hens immunized with venom. Its application for treatment of snake bite patients in Vietnam *Kiem, T.X., Long, T.X.*
Paper 4	**10:35**	Clinical features of brown snake (*Pseudonaja* species) envenoming and the brown snake paradox *Currie, B.J.*

MORNING TEA **11:00-11:30**

SYMPOSIUM 11 **11:30-13:00**

CLINICAL TOXINOLOGY II

Chair: Julian White

Paper 1	**11:30**	Struan K. Sutherland *Tibballs, J.*
Paper 2	**11:45**	Cardio-toxicity in Irukandji Syndrome: a preliminary study of sub clinical injury *Cullen, P., Carrette, T., Mulcahy, R.F., Pereira, P.L., Seymour, J.*
Paper 3	**12:00**	"Severe Irukandji syndrome" The epidemiology, management and name change? *Little, M., Pereira, P., Seymour, J., Mulcahy, R., Cullen, P., Carrette, T.*
Paper 4	**12:15**	Correlation between severity of Irukandji syndrome and nematocyst identification from skin scrapings *Huynh, T., Seymour, J., Carrette, T., Mulcahy, R., Pereira, P., Cullen, P., Little, M.*
Paper 5	**12:30**	The use of pressure immobilization bandages in the first aid management of cubozoan envenomings *Seymour, J., Carrette, T., Cullen, P., Mulcahy, R.F., Little, M., Pereira, P.L.*
Paper 6	**12:45**	*In vitro* and *in vivo* analysis of 'Jimble' jellyfish (*Carybdea rastoni*) venom *Winkel, K.D., Tibballs, J., Ross-Smith, M., Lambert, G., Lau, C., Wiltshire, C., Doube, J., Angus, J.A.*

TUESDAY, 9 JULY 2002

LUNCH **13:00-14:00**

TJAPUKAI VISIT (optional) **13:00-15:30**

WORKSHOP-1 **13:00-15:30**

CUBOZOAN WORKSHOP (optional)

Chair: Jamie Seymour

	13:00	Discussion
Paper 1	**14:00**	Mechanisms of envenoming in box jellyfish; could this explain delayed onset of symptoms in Irukandji syndrome victims? *Seymour, J.*
	14:15	Discussion

WORKSHOP-2 **14:00-15:30**

EMERGING AND RE-EMERGING ID WORKSHOP

Chair: Tim Inglis

Paper 1	**14:00**	Emerging infectious diseases of the Indian ocean rim (EIDIOR) - the EIDIOR event monitor *Inglis, T.J.J., Inglis, J.D.*
Paper 2	**14:20**	Smallpox and tularaemia: an update *Robertson, A.G.*
Paper 3	**14:40**	North Queensland Infectious Diseases - a potpouri *McBride, J.*
	15:00	Discussion

AFTERNOON TEA **15:30-16:00**

PLENARY-1 **16:00-17:30**

ACTM CONVOCATION

Chair: Robert Hirst

	16:00	**Convocation Address** Australian Toxinology - Pioneers to Frontiers *Ken Winkel*
	16:15	**Ashdown Orator** Bugs, bites and stings: clinical tropical medicine in Northern Australia *Bart Currie*

TUESDAY, 9 JULY 2002

SYMPOSIUM 12 **16:00-17:30**

SNAKE TOXINS III

Chair: Kevin Broady

Paper 1 **16:00** Structures of phospholipase A_2 genes expressed in snake pancreas and venom glands reveal the molecular evolution of toxic phospholipase A_2 genes
Fujimi, T.J., Kariya, Y., Tsuchiya, T., Tamiya, T.

Paper 2 **16:15** Composition of Russell's viper (RV) venom - an analysis of the expressed sequence tags from RV venom gland cDNA library
Nuchprayoon, I., Sai-ngam, A.

Paper 3 **16:30** Characterization of the geographic variations in venom phospholipases A_2 of Taiwanese bamboo viper (*Trimeresurus stejnegeri*)
Tsai, I.H., Chen, Y.H., Wang, Y.M., Tsai, T.S.

Paper 4 **16:45** Development of ELISA kits for species identification of venoms from four snakes of medical importance in Vietnam
Le, V.D., Gopalakrishnakone, P., Khoo, H.E.

Paper 5 **17:00** Electrospray LC-MS fingerprinting of *Acanthophis* (death adder) venoms: taxonomic and toxinological implications
Fry, B.G., Wickramaratna, J.C., Hodgson, W.C., Alewood, P.F., Kini, R.M., Ho, H., Wuster, W.

Paper 6 **17:15** Cardiovascular and haematological effects of Papua New Guinea small-eyed snake (*Micropechis ikaheka*) venom and their neutralisation with CSL polyvalent snake antivenom
Tibballs, J., Carroll, T., Fry, B.G., Hawdon, G., Sourial, M., Baker, T., Winkel, K.

SYMPOSIUM 13 **19:30-21:00**

CLINICAL TOXINOLOGY III

Chair: Bart Currie

Paper 1 **19:30** Chronic envenomation syndromes
Pearn, J.H.

Paper 2 **19:45** Deaths from snakebite in Australia 1979-2000
Hawdon, G., Rofe, G., McGain, F., Sutherland, S., Harrison, J., Winkel, K.

Paper 3 **20:00** Brown snake envenoming in Western Australia: are we giving enough antivenom?
Little, M., Yeung, J., Daly, F., Murray, L., Jelinek, G.

Paper 4 **20:15** Biodistribution of intramuscular and intravenous injection of $F(ab')_2$ antivenom
Saesow, N., Chanhome, L., Nuchprayoon, I., Wilde, H.

Paper 5 **20:30** Oral prednisone for limb edema in children with green pit viper bites: a randomized controlled trial
Nuchprayoon, I., Pongpan, C.

Paper 6 **20:45** Bites by spiders of the family Theridiidae: a prospective study of spider envenomation
Isbister, G.K., Gray, M.R.

WEDNESDAY, 10 JULY 2002

SYMPOSIUM 14 **09:00-11:00**

MICROBIAL AND MALARIAL TOXINS

Chair: Leonard Smith

Paper 1	**09:00**	Pharmacology and safety of therapeutic botulinum neurotoxin preparations *Aoki, K.R.*
Paper 2	**09:45**	Role of alpha-toxin and perfringolysin O in *Clostridium perfringens*-mediated gas gangrene *Rood, J.I., Awad, M.M., Ellemor, D.M., Boyd, R.L., Emmins, J.J.*
Paper 3	**10:10**	Structural studies of pore-forming protein toxins *Parker, M.W.*
Paper 4	**10:35**	Synthetic glycosylphosphatidylinositol as a candidate anti-toxic vaccine against malaria *Schofield, L., Hewitt, M.C., Evans, K., Siomos, M.-A., Seeberger, P.H.*

MORNING TEA **11:00-11:25**

EXCURSIONS: **11:25-17:30**
GREEN ISLAND or SKYRAIL/KURANDA

WORKSHOP-3 **20:00-21:00**

ANTIVENOM PRODUCTION WORKSHOP

Chair: Ken Winkel, Jorge Solis-Paniagua

Paper 1	**20:00**	A novel brown snake *Pseudonaja textilis* avian IgY and ovine IgG antivenom with improved efficacy against the major toxic activities *Madaras, F., Mirtschin, P.J., Almeida, A., Kuchel, T.R.*
Paper 2	**20:15**	Are polyvalent antivenoms really polyspecific? *Wuster, W., Laing, G.D., Richards, A., Theakston, R.D.G., Puorto, G., Salomao, M.G., Thorpe, R.S., Warrell, D.A.*
	20:30	Discussion

THURSDAY, 11 JULY 2002

SYMPOSIUM 15 **09:00-11:00**

APPLIED TOXINOLOGY

Chair: Paul Alewood

Paper 1 **09:00** Adding value to marine toxins
Blunt, J.W., Munro, M.H.G.

Paper 2 **09:45** Discovery, structures and insecticidal properties of the cyclotides: circular mini-proteins from plants
Craik, D.J,, Anderson, M.A., Clark, R., Daly, N.L., Jennings, C.V., Plan, M., West, J.

Paper 3 **10:10** ShK toxin as a potential diagnostic tool for relapsing-remitting multiple sclerosis
Pennington, M., Calabresi, P., Yun, S., Allie, R., Wulff, H., Beeton, C., Chandy, K.G.

Paper 4 **10:35** Deadly spider makes good - discovery, pharmacophore mapping, and commercial applications of insecticidal neurotoxins from Australian funnel-web spiders
King, G.F.

MORNING TEA **11:00-11:25**

SYMPOSIUM 16 **11:25-13:00**

ARACHNID TOXINS I

Chair: Glenn King

Paper 1 **11:25** Tanantula toxins as discovery tools for the study of acid-sensing and voltage-dependent cationic channels
Escoubas, P., Diochot, S., Lazdunski, M.

Paper 2 **11:50** Martentoxin, a distinct K^+ channel-blocking ligand: purification, genomic organization, electrophysiological and biosensor binding characteristics
Ji, Y.H., Ye, J.G., W, W.X., He, L.L., Li, Y.J., Yan, Y.P., Li, C., Tan, Z.Y., Zhou, Z.

Paper 3 **12:15** κ-hefutoxin1: a novel toxin from the scorpion *Heterometrus fulvipes* with unique structure and function
Srinivasan, K.N., Sivaraja, V., Huys, I., Sasaki, T., Kumar, T.K.S., Sato, K., Tytgat, J., Kini, R.M., Gopalakrishnakone, P.

Paper 4 **12:30** Partial protein and DNA sequences of *Latrodectus hasselti, L. hesperus* and *L. mactans* latrotoxins: are they homologous?
Graudins, A., Sung, K.L., Hains, P., Padula, M., Broady, K.W., Nicholson, G.M.

Paper 5 **12:45** Insecticidal cooperativity between amphipathic peptides and neurotoxins in spider venoms
Corzo, G., Villegas, E., Gomez-Lagunas, F., Possani, L.D., Belokoneva, O.S., Nakajima, T.

THURSDAY, 11 JULY 2002

LUNCH **13:00-14:00**

SYMPOSIUM 17 **14:00-14:30**

ARACHNID TOXINS II

Chair: Wayne Hodgson

Paper 1 **14:00** Structure-function studies of neurotoxic peptides with or without ICK motif purified from the venom of the Chinese bird spiders
Liang, S., Peng, K., Shu, Q., Liu, Z.China)

Paper 2 **14:15** Molecular characterization of the insecticidal neurotoxin J-ACTX-Hv1c
Maggio, F., Reenan, R.A., King, G.F.

IST BUSINESS MEETING **14:30-15:30**

AFTERNOON TEA **15:30-16:00**

POSTER SESSION 2 **16:00-17:30**

Chair: Bruce Livett and Graham Nicholson

Microbial, Plant, Arthropod and Marine Toxins

Poster 2-1 Some toxic and immunologic studies on *Loxosceles* venom gland homogenates from spiders of South America and North America
de Roodt, A.R., Estevez, J., Litwin, S., Dokmetjian, J.Ch., Paniagua, J.

Poster 2-2 Chemical characterization of the major components of the crude venom from the spider *Macrothele gigas* (Hexathelidae)
Corzo, G., Dai, L., Naoki, H., Haupt, J., Nakajima, T.

Poster 2-3 Structure characterization of spider toxin stored in the venom of *Nephila madagascariencis* and *Macrothele gigas*
Naoki, H., Fujita, T., Dai, L., Corzo, G., Andriantsiferana, M., Haupt, J., Nakajima, T.

Poster 2-4 The identification, characterisation and structure determination of a group of mu-agatoxin-like components from the venom of Australian funnel-web spider species
Wilson, D., Rosengren, K.J., Daly, N.L., Alewood, P.F., Craik, D.J.

Poster 2-5 Structure-function studies of the insect-specific calcium channel blocker ω-ACTX-Hv2a
Sollod, B., Wilson, D., Wang, X.-H., Reenan, R.A., Nicholson, G.M., Alewood, P.F., King, G.F.

Poster 2-6 Female wolf spider (*Lycosa* spp.) crude venom modulates [^{3}H]-dopamine release in the rat caudate putamen and may contain a myotoxic component
Ramasamy, S., Lawrence, A.J., Hodgson, W.C.

Poster 2-7 Modulation of sodium channel gating and kinetics by δ-missulenatoxin-Mb1a from the Australian eastern mouse spider *Missulena bradleyi*
Gunning, S., Khalife, A., Padula, M., Smith, R., Broady, K.W., Nicholson, G.M.

Poster 2-8 Characterization of the venom components in spider wasps
Hisada, M., Murata, K., Yasuda, A., Iwashita, T., Naoki, H., Nakajima, T.

Poster 2-9 Application of MAIDI-TOF MS and LC-MS/LC-MSMS in characterizing novel peptides in scorpion venom
Dai, L., Yasuda, A., Naoki, H., Nakajima, T.

Poster 2-10 Toxins and genes from the venom of the Asian scorpion *Buthus martensi* Karsch
Goudet, C., Chi, C-W., Tytgat, J.

Poster 2-11 Dynamic determination and possible mechanism of amino acid transmitter release from rat spinal dorsal horn induced by the scorpion venom and a neurotoxin (Bmk I)
Zhang, X.Y., Zhang, J.W., Chen, B., Bai, Z.T., Shen, J., Ji, Y.H.

Poster 2-12 The inhibitory effects of BmK IT_2 on nociceptive behavior and c-Fos expression in rat spinal dorsal horn induced by formalin and a possible mechanism
Zhang, X.Y., Bai, Z.T., Ji, Y.H.

Poster 2-13 c-Fos expression in rat spinal cord induced by scorpion Bmk venom via plantar subcutaneous injection
Bai, Z.T., Chen, B., Zhang, X.Y., Fan, G.L., Ji, Y.H.

Poster 2-14 Biosensor binding of BmK abT, a unique neurotoxic polypeptide on mammalian brain and insect sodium channels
Ji, Y.H., Wang, W.X., Wang, Q., Huang, Y.P.

Poster 2-15 Cloning of cDNAs encoding short insectotoxins from *Mesobuthus tamulus*
Newton, K., Armugam, A., Jeyaseelan, K.

Poster 2-16 Tamapin: a peptide from the venom of the indian red scorpion (*Mesobuthus tamulus*) which targets SK channels and AHP currents in central neurones
Strong, P.N., D'hoedt, D., Stocker, M., Pederzani, P., Joseph, J.S., Kini, R.M., Doorty, K.B., Wadsworth, J.D.F., Sapatnekar, S., Gadre, S.V., Jeyaseelan, K.

Poster 2-17 Expression and purification of *Clostridium botulinum* type C and D neurotoxin heavy chain subunits
Woodward, L.A., Burnell, J.N., Oguma, K., Hirst, R.G.

Poster 2-18 The *in vitro* anti-snake venom studies of polyphenols from Thai medicinal plants
Pithayanukul, P., Ruenraroengsak, P., Pakmanee, N., Bavovada, R.

Poster 2-19 Anti-necrotizing activity of tannic acid and Thai medicinal plants containing tannin against Thai cobra venom
Pithayanukul, P., Ruenraroengsak, P., Pakmanee, N., Bovovada, R.

Poster 2-20 Influence of clinoptilolite on the toxic effects of mycotoxin aurofusarin in Japanese quails
Dvorska, J.

Poster 2-21 Gene expression changes of cultured human liver cells exposed to aflatoxin B1
Zhong, S., Srinivasan, K.N., Gopalakrishnakone, P.

Poster 2-22 *Ostreopsis* sp., a possible origin of parrotfish toxin
Taniyama, S., Terada, M., Nishio, S., Takatani, T., Arakawa, O., Noguchi, T.

Poster 2-23 Forensic analysis of a victim of paralytic shellfish poisoning mediated by the Xanthid crab, *Zosimus aeneus*
Robertson, A., Llewellyn, L.E., Dodd, M.J., Ericson, G., de Koning, C., Negri, A.P.

Poster 2-24 Occurence of paralytic shellfish poison in the starfish *Asterina pectinifera* collected from Kure Bay, Hiroshima Prefecture, Japan
Ito, K., Asakawa, M., Sida, Y., Miyazawa, K.

Poster 2-25 Preliminary results of the evaluation of an immunoassay-based ciguatoxin test-kit using Australian fish
Oliver, L.M.

Poster 2-26 Venom and cnidome comparisons between 4 Australian cubozoans
Oliver, L.M., Wilce, J., Seymour, J.E.

Poster 2-27 Measuring cardiac changes in box jellyfish envenomed animal models using a vascular doppler
Carrette, T., Cullen, P., Seymour, J.

Poster 2-28 Visualization of tetrodotoxin (TTX) in the skin of two marine puffers *Takifugu vermicularis* and *Chelonodon patoca* under light microscope
Mahmud, Y., Okada, K., Takatani, T., Kawatsu, K., Hamano, Y., Arakawa, O., Noguchi, T.

Poster 2-29 Structure and membrane interactions of equinatoxin II, a β-sandwich that forms oligomeric pores in membranes
Norton, R.S., Hinds, M.G., Zhang, W., Hansen, P.E., Anderluch, G., Lam, Y-H., Bonev, B., Watts, A., Separovic, F.

Poster 2-30 Conopeptides from the venom of the Mediterranean worm hunting *Conus ventricosus*: biochemical, structural and functional characterisation of Contryphan-Vn
Raybaudi Massilia, G., Grolleau, F., Schinina, M.E., Barbier, J., Bournaud, R., Molgo, J., Ascenzi, P., Polticelli, F.

Poster 2-31 The milked venom from *Conus geographus* holds many surprises
Bingham, J.-P., Whittal, R., Semchuk, P., Moczydlowski, E.

CONFERENCE DINNER **19:30-23:00**

FRIDAY, 12 JULY 2002

SYMPOSIUM 18 **08:45-11:00**

CONOTOXINS

Chair: Ed Moczydlowski

Paper 1	**08:45**	Barnes and Southcott - two true pioneers *Pearn, J., Fenner, P.*
Paper 2	**09:00**	Conotoxins and cone snails: evolving a successful neuropharmacological strategy *Olivera, B.M.*
Paper 3	**09:45**	Analogues of endogenous neuropeptides on *Conus* venoms *Cruz, L.J., Lirazan, C.B., Jimenez, E.C., Craig, A.G., Olivera, B.M.*
Paper 4	**10:10**	Solution structure of μ-conotoxin PIIIA, a preferential inhibitor of persistent TTX-sensitive sodium channels *Lewis, R.J., Nielsen, K.J., Watson, M., Adams, D.J., Hammarstrom, A.K., Gage, P.W., Hill, J.M., Craik, D.J., Thomas, L., Adams, D., Alewood, P.F.*
Paper 5	**10:35**	Discovery of analgesic conotoxins *Alewood, P.*

MORNING TEA **11:00-11:15**

SYMPOSIUM 19 **11:15-13:05**

MARINE TOXINS I

Chair: Ray Norton

	11:15	**Address by the Honorable Peter McGauran, Minister for Science**
Paper 1	**11:30**	Conantokin-L, a new NMDA receptor antagonist from *Conus lynceus* *Jimenez, E.C., Walker, C., Donevan, S., White, H.S., Zhou, L., Cruz, L.J., Olivera, B.M.*
Paper 2	**11:45**	α conotoxins from *Conus anemone* *Sandall, D.W., Satkunanathan, N., Bingham, J.-P., Moczydlowski, E., Down, J.G., Livett, B.G., Gayler, K.R.*
Paper 3	**12:00**	Affinity chromatography on Cm-papain-sepharose as a method for purification of recombinant saxiphilin and related saxitoxin-binding proteins *Krishnan, G., Moczydlowski, E.*
Paper 4	**12:15**	Saxitoxin binding proteins in the blood: a means of detoxification or decoy receptors? *Llewellyn, L.E., Robertson, A., Robillot, C.*
Paper 5	**12:40**	Two-step membrane binding of equinatoxin II, a pore-forming toxin from the sea anemone *Actinia equina*, involves an exposed aromatic cluster and a flexible helix *Anderluh, G., Macek, P., Hong, Q., Lakey, J.H.*

FRIDAY, 12 JULY 2002

LUNCH **13:05-14:00**

SYMPOSIUM 20 **14:00-15:30**

MARINE TOXINS II

Chair: Lyndon Llewellyn

Paper 1 **14:00** Aspects of the work of Dr Robert Endean (1925-1997): Australian marine biologist and toxinologist
Hawgood, B.J.

Paper 2 **14:15** A structurally conserved K^+-channel blocking peptide in different *Conus* venoms
Mebs, D., Kauferstein, S., Tytgat, J.

Paper 3 **14:30** Ecological reasons for variations in venom lethality in two species of Australian box jellyfish, Chironex fleckeri and Chiropsalmus sp
Carrette, T., Alderslade, P., Seymour, J.

Paper 4 **14:45** Puffer fish poisoning: a poorly recognised and potentially life-threatening condition in Australia
Isbister, G.K., Son, J., Lin, C.S., Ujma, J., Smith, B., Milder, D.G., Kiernan, M.C.

Paper 5 **15:00** Identification of toxin and fish species in fraud dried mullet roe implicated in food poisoning
Hseih, Y.-W., Hwang, P.A., Pan, H.H., Chen, J.B., Hwang, D.F.

Paper 6 **15:15** "What on earth is ciguatera?"
Lewis, R.J.

CONFERENCE CLOSE **15:30-16:00**

Abstracts of Lectures

Symposium 2 - Paper 1

HEALTH CARE FOR THE POLAR TOURIST

D.Tingay

Royal Womens Hospital, Carlton, Melbourne, VIC 3052 Australia

"To anyone who goes to the Antarctic, there is a tremendous appeal, an unparalleled combination of grandeur, beauty, vastness, loneliness, and malevolence- all of which sound terribly melodramatic- but which truly convey the actual feeling of Antarctica. Where else in the world are all of these descriptions really true."
-Captain T.L.M. Sunter

Since the collapse of the former Soviet Union Antarctica has become a feasible travel destination only limited by access and cost for travellers. In the 2000/2001 southern summer season 12,285 tourists visited Antarctica[1], the majority visited the Antarctic peninsula and Ross Sea regions by ship (20 to 400 passengers each). Most tourists visit for between one and two weeks and could engage in a wide range of activities including shore landings from small inflatable craft, kayaking, camping, mountaineering, helicopter scenic flights and scuba diving. Most ships are working Russian scientific ice class vessels, although modified for "cruising" and comfortable they are far from luxurious. Most tourist vessels have an on board infirmary and "western" resident medical officer as well as a Russian medical officer. Antarctica is a unique and remote place and provision of health care problematic and well documented[2]. Unfortunately very little is known about the exact health needs of polar tourists. Tourists are generally older but active and embrace the "adventure" of polar cruises. Polar tourism health care needs to be a mixture of general practice, remote wilderness medicine, cruise ship medicine, travel medicine (especially as most tourists embark from South America), public health and occupational medicine. A lack of definitive data on the health care needs of polar tourists makes supplying on board infirmaries difficult. Intensive care and evacuations are hampered by weather, ice conditions, remoteness and supply issues and, if feasible, can take up to 72 hours. Deaths on board polar tourist vessels have been well documented[3]. Pre-departure screening and education of prospective passengers for what is likely to be an experience of a life time is of paramount importance and relies heavily on the passengers physician. The on board medical officer needs a wide range of skills, apart from any medical problems they may be called on as dentist, safety officer, tour guide, public relations duties and zodiac driver. Most medical officers are employed directly by the tour operator and balancing the expectations and interests of the patient, doctor, other passengers, guides and tour operator can place the doctor in a challenging situation. The author's experiences on board a polar tourist vessel over the 2001/2002 southern summer season will be presented to illustrate the diversity of polar tourist health care, especially the issue of the elderly in remote and hostile environments.

[1]International Association of Antarctica Tour Operators data 2002.
[2]Lugg DJ. (2000) *JAMA* **283**, 2082-2084.
[3]Lamberth P. (2001) *MJA* **175**, 583-4

Symposium 2 - Paper 2

TRAVEL MEDICINE - THE VIEW FROM EVEREST

John Heydon

Himalayan Trust/ University of Otago

This presentation is based on 2 1/2 years working at Kunde Hospital in Nepal.

Mt Everest has been a lure to Westerners for a century. The first explorers into the area were followed by mountaineers. After Mt Everest was climbed by Sir Edmund Hillary in 1953, and then Nepal was opened up to foreigners interest in both Everest and the wider Himalayas surged. Nowadays many climbing expeditions and trekkers travel to the Himalayas. As adventure tourism has grown two areas of Nepal, the Annapurna circuit and the Everest region (the Sagarmatha National Park), have increased steadily in popularity.

Almost 25,000 trekkers now head into the Khumbu region, where Mt Everest is, each year. Trekking has become a major source of employment and income for the Sherpa people of the Khumbu region.

The area is very high, cold and harsh. Although light aircraft have made it easy to reach Lukla, the gateway to the Khumbu, the area after that is accessed on foot along mountain trails. There is no vehicular access. For the local people life in the Khumbu is harsh; for the tourists it presents many challenges, for which many are unprepared. When ill it can be much harder to get out than it was to get in.

This talk looks at issues of pre-travel advice and common health problems of tourists, which are mainly diarrhoeal illness, respiratory problems, altitude problems, and fatigue. In addition it will outline the wider range of health problems seen amongst tourists. It will also describe some of the health problems of the Sherpa people. Although a very wide range of conditions is seen at Kunde Hospital in the Sagarmatha National Park, the 4 commonest are disorders of the gastro-intestinal tract, respiratory tract, skin and trauma.

Symposium 2 - Paper 3

HEALTH PROBLEMS IN BOUGANVILLE - PARADISE LOST?

Dagmara Poprawski

Bouganville has been trying for independence from Papua New Guinea (PNG) for many years. This has amounted to gorilla warfare with some 20,000 people lost on both sides due to the conflict.
Since 1998, the United Nations has sent in its Peace Monitoring Team in order to help the process. The team is international, and has a health section in its make up.

A number of issues will be covered in the presentation, which include public health issues of the island, main health problems and causes of death, and the health infrastruture of the island. There will also be a short coverage of the main mission of the health team and the tasks performed in humanitarian aid, and the risks to the international trvcellers to war ravaged Pacific nations.

Future directions of both local and international attempt in setting up health infrastruture will be described. Problems in this campaign will be covered ranging from political uncertainly, to education paucity, and the need for ongoing financial aid.

Symposium 2 - Paper 4

TARGETING VECTOR BEHAVIOUR AND CHARACTERISTICS FOR EFFECTIVE MALARIA CONTROL

D. N. Durrheim[1] and J. M. Govere[2]

[1] *School of Public Health and Tropical Medicine, James Cook University, Australia*
[2] *CDC, Mpumalanga, South Africa and WHO-Afro, Harare, Zimbabwe*

Malahlapanga, a remote spring in the Kruger National Park, South Africa, has proven a valuable site for seminal research on the behaviour of *Anopheles arabiensis*, the most important malaria vector in southern Africa. Research findings have been directly applied for improving malaria prevention and control.

This unique oasis supports a large perennial population of *An. arabiensis* mosquitoes. Although adult *An. arabiensis* are readily collected biting humans, they are usually obliged to feed on wild mammals that are abundant in the area, as the nearest human habitation is more than 9 km distant and the area is inaccessible to tourists. No other members of the *An. gambiae* complex are present and *An. arabiensis* mosquitoes are free of known human pathogens, including *Plasmodium spp.*

Studies conducted at Malahlapanga have found that the peak biting activity of *An. arabiensis* occurs during the predawn period and that 81% of bites on humans occur on the ankles or feet. Wearing closed shoes or application of small doses of N,N-diethyl-M-toluamide containing insect repellent to the feet and ankles dramatically reduces vector contact.

Operational research findings from Malahlapanga have influenced national guidelines, directed larviciding around residential camps in nature reserves, been employed for malaria outbreak response and offer great potential for cost-effective personal protection against *An. arabiensis* in low incidence malaria areas.

Symposium 3 - Paper 1

FROM SNAKE BITE TO SUMMIT: THE 2001 SOUTH AUSTRALIAN EVEREST EXPEDITION

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"In my state of spiritual abstraction, I no longer belong to myself and to my eyesight. I am nothing more than a single, narrow gasping lung, floating over the mists and the summits." - Reinhold Messner 1979

Animal bites and stings are one of the hazards associated with the activities typical of the globally emergent 'ecotourism' industry and wilderness based sports. In addition to the potential severity of the injury itself, the patient and/or treating physician are faced with the challenge of managing the event in a remote and/or physically threatening environment. As with so many other facets of wilderness medicine, forward planning is of utmost importance. In addition to the acute toxicity and/or trauma specifically associated with venomous bite or sting injuries, the longer-term sequelae from that encounter needs to be considered in counseling the patient. We present the unique case of a previous well, 30-year old high altitude climber who suffered coagulopathy after a snakebite in the Australian Alps. The patient undertook this climb as part of his preparation for an attempt on the north face of Mt Everest, only days prior to departure for Nepal then Tibet. His case and others will be discussed to illustrate the effects of extreme altitude on the human body. Climbing Mt Everest (8850m) is an enormous undertaking physically, mentally and logistically which many attempt but few succeed. Medically, high altitude mountaineering is an exercise in hypobaric hypoxia. Extremely high altitude is defined, as the point were effective acclimatisation to hypobaric hypoxia ceases to occur and deterioration begins. Although this will vary between individuals, it is generally defined as 5800m above sea level. The most important physiological response to high altitude is to modify the oxygen cascade, for example via hyperventilation, improved diffusion and shifts in the oxygen dissociation curve, to allow more efficient delivery of oxygen to tissues. Important responses occur within the brain and renal systems as well. Unfortunately there is no safe way of predicting who will tolerate altitude well and who will develop altitude related illnesses, although it is clear that rate of ascent and individual variability play a major role. The barometric pressure on the summit of Mt Everest is 253 mmHg, resulting in an inspired P_{O2} of only 53 mmHg. Exercise at these altitudes places enormous demands on the oxygen cascade because of reduced oxygen uptake in the setting of reduced inspired P_{O2}. Any acute injury to important muscle groups has the potential to greatly hamper a climbers performance at high altitude where even slight changes can result in major impairment. The two base camps for Mt Everest can be a busy place for a doctor with over 600 people living on the mountain each season. The scope of illnesses is wide and not solely related to altitude exposure. Although few Australians will venture above 6000m, over 10000 Australians visited Nepal in 2001 and many more visit high altitude regions in other places. Thus those providing travel medicine services should not underestimate the potential health impact of high altitude on the Australian traveller.

Symposium 4 - Paper 1

PROTEIN ENGINEERING AND THE EVOLUTION OF A RICIN VACCINE

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The ribosome-inactivating proteins (RIPs) from plants are RNA *N*-glycosidases that depurinate eukaryotic ribosomes and, in so doing, arrest protein synthesis by rendering their 60 S subunit unable to bind the elongation factor 2. Type I RIPs are single-chain proteins with molecular masses of ~30 kDa, while type II RIPs have two subunits (designated A and B) linked by a disulphide bond and are ~ 60 kDa in size. The B subunit has lectin properties and binds to galactosyl-terminated receptors on cell surfaces. The B subunit enables type II RIPs to enter cells, thus targeting the enzymatic A chain to its RNA substrate. It is this ability of the type II RIPs to translocate and be active inside cells that imparts their high toxicity in animals. Four different plant families are known to produce dichain toxins such as ricin, abrin, viscumin, and modeccin (also volkensin) with i.p. MLD_{50} ranging from 0.6 – 2.6 μg/kg. Because of their wide availability, severe pulmonary and systemic toxicity, and potential use as biological warfare/terrorism agents, USAMRIID is involved with developing prophylactic countermeasures to these types of toxins. Our discovery track for developing a ricin vaccine has progressed from a toxoid vaccine, a partially-deglycosylated subunit vaccine, to a novel recombinant subunit vaccine. This presentation will examine the development of the ricin vaccine candidate, noting where regulatory issues involving product quality, safety, and effectiveness have collided with science, and how the use of protein engineering technologies have been used to harmonize the two.

Symposium 4 - Paper 2

Global gene expression profiling of Human Genome following exposure to toxins – emerging field of TOXINOGENOMICS

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An essential use of genome sequence data is to examine the individual functions of predicted ORF's within the genome and the relationships between genes at the expression level efficiently and systematically. Many existing methods such as northern blotting, RNAse protection assay and others are very limited and do not provide sufficient throughput effectively to exploit the genomic data.

DNA microarray is also known as DNA chips, are simply glass surfaces bearing orderly arrangements of thousands of DNA fragments at discrete places, on which the fluorescent labeled DNA or RNA are ready for hybridization. This is the most powerful tool for monitoring the simultaneous expression of many thousands of genes and to large-scale gene discovery in many different organisms (Global gene expression profiling) . Human brain and liver cell culture were exposed to toxins like, Candoxin (3 finger toxin from *Bungarus candidus*) Heifutoxin (potassium channel toxin from scorpion *Heterometrus fulvipes*), tetrodotoxin (sodium channel blocker from puffer fish), aflatoxin B (fungal toxin with hepatotoxicity) and domoic acid. Gene expression profiling after exposure to these toxins, the genes which are over expressed (upregulated), under expressed (down regulated) and which are not affected were identified. Gene clustering was also done with possible insight into the mechanism/s of actions of these toxins at molecular level.

Symposium 4 - Paper 3

How do snake neurotoxins block nicotinic acetylcholine receptor?

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Snake neurotoxins are potent blockers of nicotinic acetylcholine receptors. To understand how they achieve this function, we have elaborated an experimentally based model of the α-cobratoxin-α•7 receptor complex. This was achieved using (i) a 3D model of the α•7 extracellular domain derived from the crystallographic structure of the homologous acetylcholine binding protein; (ii) the previously solved X-ray structure of the toxin and (iii) nine pairs of residues identified by cycle-mutant experiments to make contacts between the α•-cobratoxin and α•7 receptor. Since the receptor loop F occludes entrance of the toxin binding pocket, we submitted this loop to a dynamics simulation and selected a conformation that allowed the toxin to reach its binding site. The 3D structure of the toxin-receptor complex model was validated *a posteriori* by an additional double mutant experiment. The model shows that the toxin interacts perpendicularly to the receptor axis, in an equatorial position of the extracellular domain. The tip of the toxin central loop plugs into the receptor between two subunits, just below the functional receptor loop C, the C-terminal tail of the toxin making adjacent additional interactions at the receptor surface. The receptor establishes major contacts with the toxin by its loop C which is assisted by principal (loops A and B) and complementary (loops D, F and 1) functional regions. This model explains the antagonistic properties of the toxin toward the neuronal receptor and opens the way to the design of new antagonists.

Fruchart-Gaillard C. *et al.*, 2002, *Proc Natl Acad Sci* U S A. Mar 5;99(5):3216-21.

Symposium 4 - Paper 4

ANIMAL VENOMS PROTEOMICS

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The term "proteome" refers to the total protein profile of a cell or a tissue at a given time. Proteomics is most widely known as a post-genomic science, in which the protein profile of a biological sample is compared to the accumulated nucleic acid-based knowledge from the genome projects. In a typical experiment, the biological sample is analysed by 2D-PAGE, and the stained protein spots are digested in-gel by a specific enzyme such as trypsin. It is usually possible to extract a substantial portion of the proteolytic fragments from the gel and to submit them to a mass spectrometry peptide mapping. This is often completed with additional MS/MS fragmentation of the major signals to generate additional partial sequence information. The peptide mass maps thus obtained allow fast automated matching against protein databases.

Unfortunately, few genomic data are available from the venom glands; the reason for this is the biodiversity of the hundreds of thousands of living venomous animal species, each with its specific venom made of dozens or hundreds of constituents. This means that high throughput partial mapping strategies through database mass matching, although extremely useful for specific applications, can lead to confusing results and are not well adapted to venom protein characterisation. It is thus essential to distinguish between protein identification through genomic databases matching of partial peptide mass fingerprints and protein characterisation through complete *de novo* MS/MS and/or Edman sequencing of unknown compounds.

Proteomic strategies applied to animal venoms will be illustrated through several distinct examples. We have developed several novel approaches not necessarily based on 2D-PAGE sample preparation, which is not well adapted to peptides, proteins below 10 kDa or basic compounds. The direct analysis of crude venoms using on-line LC-ES-MS, MS/MS and MALDI-TOF-MS in conjunction with related micro- or nano-technologies has been used extensively in our laboratory. These techniques will be illustrated with several examples such as chemotaxonomic applications, the proteomic analysis of crude conus and scorpion venom samples (which we refer to as "venomics") leading to a full mapping of their toxin profiles (which we refer to as "toxinome"), the discovery of novel sarafotoxins (endothelin-type peptides) or bradykinin-potentiating peptides (BPP's) from snake venoms, and the study of the crude honey-bee (*Apis mellifera*) venom. The quality and validity of the results that can be obtained from classical 2D-PAGE approaches will also be discussed.

Symposium 5 - Paper 1

PROTHROMBIN ACTIVATORS FROM AUSTRALIAN SNAKE VENOMS

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Several procoagulants from snake venoms target prothrombin activation, a key step in the coagulation cascade. These snake venom prothrombin activators are classified into four groups based on their cofactor requirements. Groups A and B prothrombin activators are metalloproteinases whereas groups C and D are serine proteinases. So far, groups C and D prothrombin activators are found only in Australian snake venoms. These prothrombin activators act functionally equivalents of mammalian coagulation factors. We recently initiated studies to determine structural characteristics of these two groups of prothrombin activators. Group D prothrombin activators, such as trocarin D and hopsarin D from *Tropidechis carinatus* and *Hoplocephalus stephensi* venoms, respectively, are structurally similar to mammalian coagulation factor Xa. They have two chains; the light chain contains a gla domain and two EGF domains whereas the heavy chain contains serine proteinase domain. Group C prothrombin activator, Pseutarin C from *Pseudonaja textilis* venom, is structurally similar to mammalian coagulation factor Xa-Va complex. The nonenzymatic subunit of pseutarin C has similar domain architecture of mammalian coagulation factor Va; the heavy chain contains A1 and A2 domains whereas the light chain contains A3, C1 and C2 domains. The catalytic subunit is structurally similar to group D prothrombin activators and mammalian coagulation factor Xa. Structure-function relationships of group C and D prothrombin activators contribute significantly to our understanding of molecular details of the prothrombinase complex formation.

Symposium 5 - Paper 2

INVESTIGATION OF THE STRUCTURE, DYNAMICS AND FOLDING OF SNAKE VENOM CARDIOTOXINS

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Cardiotoxins isolated from the Taiwan cobra venom (*Naja naja atra*) are small molecular weight proteins (~ 7 kDa), containing four disulfide bonds. To-date, different cardiotoxin isoforms (CTXI, CTXII, CTXIII, CTXIV and CTX V) have been isolated from the venom of *Naja naja atra*. The three-dimensional structures of all the five cardiotoxin isoforms have been solved by multidimensional NMR techniques. Critical comparison of the structures of cardiotoxins reveal a common structural feature responsible for the lethal activity of cardiotoxins. Although the cardiotoxins show very high structural homology they exhibit significant differences in their lethal potencies. The observed differences in the lethal potencies are found to depend on the degree of exposure of the positive charge of an invariant lysine.

Backbone dynamics of CTXIII has been studied by carbon–13 relaxation meaurements at natural abundance. The overall rotational correlation co-efficient of the protein has been estimated to be 4.8 ns. Most of the residues in CTXIII have been observed to exhibit fast (τ_e < 30 ps) restricted motions (S^2 = 0.79 – 0.89). The functional important residues located at the tips of three loops are relatively flexible.

The structural stability of CTXIII has been probed by hydrogen-deuterium exchange monitored by NMR spectroscopy. Among the five beta strands in the toxin, beta strand III is found to constitute the stability core. The stability of the triple stranded beta-sheet domain is markedly higher than that of the double stranded beta-sheet domain. The refolding of CTXIII monitored by a variety of biophysical techniques reveals that the toxin refolds completely within a time span of 200 milliseconds. The chronology of the folding events in CTXIII monitored by quenched-flow H/D exchange shows that the triple-stranded beta-sheet domain folds faster than the double stranded beta-sheet domain. These results would be elaborately discussed

Symposium 5 - Paper 3

A PEPTIDE DERIVED FROM THE PHOSPHOLIPASE A_2 INHIBITOR FROM *PYTHON RETICULATUS* (PIP) EFFECTIVELY PROTECTS KAINATE-INDUCED EXCITOTOXIC NEURONAL INJURY IN RAT HIPPOCAMPAL SLICES

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From the primary structure of PIP, an endogenous phospholipase A_2 inhibitor from *Python reticulatus* serum, we have identified a peptide sequence that can strongly inhibit phospholipase A_2 activity. The 17-residue synthetic peptide, P-NT.II is able to inhibit the enzyme activities of the snake venom $sPLA_2$ (crotoxin B) and the recombinant human cytosolic $cPLA_2$ as well as that of the unpurified synovial enzyme of patients with arthritis. P-NT.II inhibits $sPLA_2$ as effectively as 12-epi-sclaradial with 5-fold selectivity over $cPLA_2$ (IC_{50} 4.9 *vs* 23.9 μM). Using this $sPLA_2$-selective inhibitor, we were able to examine the relative contributions of $sPLA_2$ and $cPLA_2$ in the cytosolic rat brain fractions before and after injection of an excitotoxin, kainic acid, and showed that $sPLA_2$ was as important as $cPLA_2$ in mediating ischemic and oxidative injuries in the rat brain. P-NT.II also exhibits potent neuroprotective activity when used in organotypic hippocampal slice cultures. Addition of P-NT.II (10 μM) or 12-epi-sclaradial (20 μM) to cultured slices before kainate application prevents the decrease in GluR 1 immunoreactivity, indicating protective capacity of the peptide to kainate-induced neural injury. The experimental evidence for the direct and selective binding of the active biotinylated-P-NT.II to $sPLA_2$ and a relatively weaker binding to $cPLA_2$ was demonstrated by ELISA. These observations provide evidence for a role of group II $sPLA_2$ in kainate-induced neuronal injury, and highlight a potential role for inhibitors of $sPLA_2$ in inhibiting excitotoxic brain injury.

Symposium 5 - Paper 4

SNAKE α-NEUROTOXIN BINDING SITE ON THE EGYPTIAN COBRA (*Naja haje*) ACETYLCHOLINE RECEPTOR IS MASKED BY GLYCOSYLATION

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Evolutionary success requires that animal venoms are targeted against phylogenetically conserved molecular structures of fundamental physiological processes. Species producing venoms must be resistant to their action. Venoms of Elapidae snakes contain α-neurotoxins, represented by the long-chain α-neurotoxin *Bungarus multicinctus* α-bungarotoxin (α-BTX) targeted against the nicotinic acetylcholine receptor (nAChR) of the neuromuscular junction. The model which presumes that cobras (*Naja* spp., Elapidae) have lost their binding site for conspecific α-neurotoxins because of the unique amino acid substitutions in their nAChR polypeptide backbone *per se* is incompatible with the evolutionary theory that (1) the molecular motifs forming the α-neurotoxin target site on the nAChR are fundamental for receptor structure and/or function, and (2) the α-neurotoxin target site is conserved among Chordata lineages. To test the hypothesis that the α-neurotoxin binding site is conserved in Elapidae snakes and to identify the mechanism of resistance against conspecific α-neurotoxins, we cloned the ligand binding domain of the Egyptian cobra (*Naja haje*) nAChR α subunit. When expressed as part of a functional *Naja*/mouse chimeric nAChR in *Xenopus* oocytes, this domain confers resistance against α-BTX but does not alter responses induced by the natural ligand acetylcholine. Further mutational analysis of the *Naja*/mouse nAChR demonstrated that an *N*-glycosylation signal in the ligand binding domain that is unique to *N. haje* is responsible for α-BTX resistance. However, when the *N*-glycosylation signal is eliminated, the nAChR containing the *N. haje* sequence is inhibited by α-BTX with a potency that is comparable to that in mammals. Preliminary data indicates that the same *N*-glycosylation also interferes with the inhibitory action of the short-chain α-neurotoxin, *Laticauda semifasciata* (Hydrophiidae) erabutoxin a. We conclude that the binding site for conspecific α-neurotoxin in Elapidae snakes is conserved in the nAChR ligand binding domain polypeptide backbone *per se*. This conclusion supports the hypothesis that animal toxins are targeted against evolutionarily conserved molecular motifs. Such conservation also calls for a revision of the present model of the α-BTX binding site. The approach described here can be used to identify the mechanism of resistance against conspecific venoms in other species and to characterize toxin-receptor coevolution.

Symposium 5 - Paper 5

STRUCTURE/FUNCTIONAL STUDY OF JARARHAGIN USING RECOMBINANT FRAGMENTS AND MONOCLONAL ANTIBODIES

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Jararhagin, a 52 kDa multidomain hemorrhagin, is found together with jararhagin-C, a 28 kDa autolysis subproduct, in *Bothrops jararaca* venom. Jararhagin comprises a metalloproteinase (M), an ECD-disintegrin (D) and a cysteine-rich (C) domain, while jararhagin-C corresponds to the last 2 domains (D/C). The M domain due to its catalytic activity, disrupts the extracellular matrix and is responsible for the severe hemorrhagic condition observed after envenoming, while the D/C domains block the $\alpha_2\beta_1$ integrin, inhibiting collagen dependant platelet aggregation. It is known that the D/C domains enhance the hemorrhagic activity of metalloproteinases. At the same time the M domain seems to participate in the inhibition of platelet aggregation. To further understand this structure/functional interaction we have cloned and expressed GST-fusion fragments, 3 from the D domain (JD49, JD89, JD98) and 4 from the C domain (JC63, JC76, JC103, JC116). For the M domain we used BAP1, a metalloproteinase comprising just the catalytic domain, from *B. asper*. JD49 corresponds to the C-terminal portion of the D domain and was chosen after alignments with short RGD-disintegrins, while the other fragments were based on Catrocollastatin-C disulfide bond pattern (1) which has 97% of identity with Jararhagin-C. JD89 and 98 comprise the D domain with the ECD-cysteine free and disulfide-bonded, respectively. JC116 corresponds to the whole C domain and the other JCs represent clusters based on disulfide bonding. A series of 7 murine monoclonal antibodies (MAJar1 to 7) raised against native jararhagin were tested with the fragments in dot blot tests. Our results show that JC fragments were not recognized by the MAJars, even more, they were weakly recognized by anti-jararhagin polyclonal antibody, suggesting that this portion must be hidden in the native molecule. MAJar2, 6 and 7 recognized JD89 and 98, but not JD49, demonstrating their interaction with the N-terminal portion of the D domain. MAJar1 recognized all JD fragments, including JD49, suggesting its interaction with the C-terminal portion of the D domain. MAJar4 and 5 may be binding zones of interaction between the D and C domains since they failed in recognizing any of the fragments, although they interact with jararhagin-C. MAJar3 recognized both, JD49 and BAP1, demonstrating an spatially close interaction between the M and the C-terminal portion of the D domain. Moreover, MAJar3 is the only one which completely neutralized the hemorrhage caused by jararhagin. This fact suggests that this interaction must be at the catalytic level and probably with the ECD motif.

1. Calvete *et al.* (2000) *Protein Science* **9,** 1365-73.

Symposium 6 - Paper 1

MOLECULAR EPIDEMIOLOGY OF MELIOIDOSIS IN AUSTRALIA

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Melioidosis is endemic in northern Australia and the Darwin prospective study has documented over 350 culture-confirmed cases of melioidosis over the last 12 years. Various molecular methods have been used to elucidate the epidemiology of melioidosis. These include ribotyping, random amplified polymorphic DNA analysis (RAPD) and pulsed-field gel electrophoresis. All three methods have shown considerable diversity amongst *Burkholderia pseudomallei* isolates from the Top End of the Northern Territory. In contrast, clonality was found amongst isolates from animal cases and a human case spanning 25 years on neighbouring farms in south-west Western Australia. This suggested introduction of *B. pseudomallei* to that temperate location via animals imported from the north.

Molecular typing has confirmed that human or animal *B. pseudomallei* isolates can be identical to epidemiologically related environmental (soil or water) isolates. As also documented from a northern Western Australia outbreak of melioidosis, we have shown a cluster of cases and fatalities in a remote Northern Territory Aboriginal community to be linked to contamination of the community water supply with *B. pseudomallei.* The water supply is unchlorinated and these are apparently the first documented deaths for decades in Australia from infection acquired from potable water.

Isolates of *B. pseudomallei* from cases of recurrent melioidosis have confirmed the vast majority to be relapse of the original strain rather than new infection. Associations of clinical presentations (eg neurological melioidosis), severity and outcomes of melioidosis with particular molecular types have yet to be elucidated.

Symposium 6 - Paper 2

CATCHING A TIGER BY THE TAIL: IN VITRO IDENTIFICATION OF POTENTIAL VACCINE CANDIDATES FOR MELIOIDOSIS

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There is currently no licensed vaccine for the potentially fatal infection known as melioidosis. Recent interest has focussed on the flagellar organelle of *Burkholderia pseudomallei*, the bacterial cause of melioidosis. In a previous study it was noted that *B. pseudomallei* penetration of an amoebic trophozoite model of infection began with flagellar adherence to the amoeba surface. A *B. pseudomallei* flagellum-negative mutant was used to demonstrate a requirement for an intact flagellar apparatus before cellular invasion could take place. This cellular model of infection was then used to test the effect of purified flagellin, anti-flagellin antibodies, a flagellin-capsular polysaccharide glycoconjugate and an antibody to the conjugate. Flagellin and the corresponding antibody preparation did not significantly alter cellular outcomes in the amoeba model. The glycoconjugate and matching antibody preparation did alter the course of cellular infection at higher concentrations. These results provide preliminary evidence to implicate the flagellum and the capsular polysaccharide in *B. pseudomallei* cellular invasion and thus identify these two components as potential vaccine candidates.

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Symposium 6 - Paper 3

G-CSF IN THE TREATMENT OF SEVERE MELIOIDOSIS; RECENT DEVELOPMENTS

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Granulocyte-colony stimulating factor (G-CSF) has been shown in *in vitro* studies and in animal models to improve both the number and function of neutrophils. Impaired intracellular killing by neutrophils is felt to be important in the pathogenesis of melioidosis. Risk factors for melioidosis, including diabetes, chronic renal failure and alcoholism, have been shown to impair neutrophil function. However, clinical trials of G-CSF in other causes of sepsis have generally been disappointing, although the intracellular location of *Burkholderia pseudomallei* may be a key difference with other causes of septic shock. We have demonstrated the central role of G-CSF in the pathogenesis of melioidosis by the use of G-CSF gene knockout mice.

G-CSF was adopted in the routine treatment of septic shock at Royal Darwin Hospital in December, 1998 in an attempt to reduce the high mortality associated with this condition. Since that time, G-CSF has been administered to 20 patients with melioidosis and septic shock, with a mortality of 10% in this group. Prior to the introduction of G-CSF, the development of septic shock associated with melioidosis (n=20) was associated a 95% mortality. However, this apparent benefit may be confounded by a number of factors, including the appointment of a full-time intensivist as the Director of the Intensive Care Unit. During this time, other changes instituted include the adoption of standardized treatment protocols, the early use of enteral feeding, the early use of meropenem and the use of vasopressin and physiological dose steroids.

The use of routine G-CSF at RDH has been associated with a dramatic fall in mortality from severe melioidosis. We intend to pursue *in vitro* models, animal studies and clinical trials to confirm this apparent benefit.

Symposium 6 - Paper 4

G-CSF IMMUNOTHERAPY FOR TREATMENT OF ACUTE DISSEMINATED MURINE MELIOIDOSIS

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Burkholderia pseudomallei, the causative agent of melioidosis has emerged as an important pathogen in tropical regions of Australia and Southeast Asia. Mortality rates of up to 70% are still observed in some areas. We have developed a murine model for melioidosis that allows novel treatment approaches to be investigated. The progression of disease in BALB/c mice is rapid, resulting in septicaemic shock and death within 96 hours of infection. This resembles acute septicaemic human melioidosis. Using this model we have successfully used selected antibiotics to improve clinical outcome in BALB/c mice, following infection with a normally lethal dose of virulent *B. pseudomallei*. It has been proposed that adjunctive immunotherapy using G-CSF combined with antibiotics may provide an alternative approach to chemotherapy alone, which can be ineffective in patients with acute melioidosis. This study looked at the potential for G-CSF therapy both alone and as an adjunct in the treatment of acute disseminated *B. pseudomallei* infection in BALB/c mice. Six groups of BALB/c mice were used and infected with a virulent strain of *B. pseudomallei*. Therapeutic variables studied were G-CSF alone, ceftazidime alone, both G-CSF and ceftazidime, pre-treatment with G-CSF, pre-treatment with both G-CSF and ceftazidime and an infected, untreated control. Surviving mice were sacrificed and splenic bacterial loads were determined. Combining G-CSF with ceftazidime offered no advantage over ceftazidime alone. Pretreatment with G-CSF however did offer a significant benefit.

Symposium 6 - Paper 5

The efficacy of *Clostridium botulinum* type C and D neurotoxin subunit vaccines evaluated in mice

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Botulism is an intoxication caused by neurotoxins (BoNT) produced by the bacterium *Clostridium botulinum.* Seven antigenically distinct neurotoxins have been identified, designated BoNT/A–G, of these only BoNT/C and D have been associated with botulism in cattle in Australia. To combat the disease, a bivalent vaccine consisting of formalin treated type C and D toxins is currently available. Although efficient, several problems associated with it's production have been identified. These include a long lead time for production, safety considerations due to the use of active toxin and a heavy reliance on animal testing, Such problems could be overcome through the use of a subunit vaccine.

The 50 kDa carboxy terminal end of the neurotoxin heavy chain (H_C) has been shown to contain the major determinants for toxin binding (1) and is able to elicit a protective immune response when used to vaccinate mice (2-4). In this study several H_C subunit proteins of BoNT/C and D were expressed and evaluated as candidate immunogens. To determine vaccine efficacy, mice were vaccinated with a range of doses and challenged with $10 - 10^5$ LD_{50} of toxin. For both subunit proteins, C50 and D50, two doses of 10 μg afforded partial protection against homologous toxin challenge of up to 10^5 LD_{50}. A bivalent vaccine, consisting of mixture of these two proteins was also investigated and provided protection against both BoNT/C and D challenge. Serum antibody levels were determined by both ELISA and serum neutralisation assays. ELISA titres correlated well with survival after toxin challenge. For monovalent vaccines, all mice with an anti-C50 titre of >160 and 78% of mice with an anti-D50 titre of >160 survived challenge with up to 10^3 LD_{50} of the homologous toxin. The serum neutralisation assay did not correlate as well with survival after toxin challenge. Further vaccine trials are being undertaken in cattle.

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Symposium 7 - Paper 1

PIONEERS IN AUSTRALIAN TOXINOLOGY: FROM JOSEPH BANKS TO CHARLES KELLAWAY

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The studies presented at this conference extending the frontiers of knowledge inevitably draw on historic research patterns and individual personalities for inspiration. This presentation will briefly revisit some of the early observations and investigations of Australia's venomous fauna as a prelude to later presentations on particular personalities and their impact on Australian toxinology. It will begin with a review of the eighteenth century encounters between the scientists and sailors of HMS Endeavour and Resolution with our venomous and toxic creatures: "Of insects here [in New South Wales] were but few sorts and among them only the Ants were troublesome to us...their courage if possible excells their industry; if we accidentally shook the branches on which such nest were hung thousands would immediately throw themselves down, many of which falling upon us made us sensible of their stings and revengefull dispositions..."[1] The European fascination and horror of Australia's venomous creatures was considerably magnified by the rarity of fatal misadventures. This led to a tendency, still evident today, to endow these animals with almost supernatural powers: "A correspondent for the Melbourne Record states that he is acquainted with a person who has been cured of epilepsy by taking three teaspoons of rum in which the heads of black snakes have been infused for ten days. The influence of the imagination is so great that this remedy might be worthy of a trial in nervous epilepsy."[2] Similarly confused were practices relating to the management of snake bite: "A labouring man...having been bitten on the hand by a snake..took a pistol, loaded it with powder and a few small shot, and blew a hole through his hand...it bled profusely, and most certainly stopped the effects of the poison, but as might have been supposed, the remedy was worse than the disorder."[3] Eventually, as Pasteur, Behring and Ehrlich developed modern microbiology and immunology, so too did the discipline of toxinology develop to debunk dangerous mythology of the past. Whilst the work of Martin and Tidswell in the late nineteenth century showed the early rigour of our toxinologists, little progress was made until the time of Kellaway and Hamilton-Fairley, the latter charged by his experiences of snake bite in India: "Collaborating with Fairley, Kellaway studied the modes of action of venom milked from captured Australian snakes - tiger, black and brown. They discovered that each variety had its selective mode of action...Moreover they appreciated the potential value to man of snake antivenines and, with the cooperation of the Commonwealth Serum Laboratories, a tiger snake antivenine was made in horses."[4] Thus, standing on the shoulders of giants, did the modern era of Australian toxinology begin.

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Symposium 7 - Paper 2

ISOLATION AND PHARMACOLOGICAL CHARACTERISATION OF A MYOTOXIC PLA_2 FROM A DEATH ADDER VENOM

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Death adders (genus *Acanthophis*) belong to the Elapidae family of snakes. To date, twelve species and three sub-species have been described. Death adders are found not only in continental Australia, but North throughout the Torres Straight Islands, Papua New Guinea, Irian Jaya and the Indonesian islands of Seram, Halmahera, Obi and Tanimbar. It has long been thought that death adder venoms are devoid of myotoxic activity based on studies done on *A. antarcticus* venom (1,2). However, a recent clinical study reported rhabdomyolysis in patients following death adder envenomations in Papua New Guinea by a species thought to be different to *A. antarcticus* (3). Consequently, this study examined *A. rugosus* (Irian Jayan death adder) venom for myotoxic activity, and isolated the first myotoxin from a death adder venom. In addition, we examined the effectiveness of CSL death adder antivenom, which is raised against *A. antarcticus* venom, in neutralising the myotoxic activity.

A. rugosus venom was fractionated by RP-HPLC using a Jupiter C18 column. Molecular weights and purity of fractions were determined by mass spectrometric analysis using a PE-SCIEX triple quadrupole mass spectrometer. Purified components were N-terminally sequenced. Isolated components were screened for myotoxicity using the chick directly (0.1Hz, 2ms, supramaximal V) stimulated biventer cervicis nerve-muscle preparation (CBCNM) in the presence of d-tubocurarine (10 μM). Those that caused a significant contracture of skeletal muscle (i.e. a rise in baseline) and/or inhibited the direct twitches were considered myotoxic. This was confirmed by histological examination of CBCNM.

High PLA_2 activity was detected in both *A. rugosus* venom (140.2 ± 10.4 μmol/min/mg) and acanmyotoxin-1 (153.4 ± 11 μmol/min/mg). Both *A. rugosus* venom (10-50 μg/ml) and acanmyotoxin-1 (MW 13811; 10^{-7}-10^{-6} M) caused dose dependent inhibition of direct twitches and increase in baseline tension. In addition, dose dependent morphological changes in skeletal muscle were observed. Prior incubation (10 min) of CSL death adder antivenom (5 units/ml) or inactivation of PLA_2 activity with 4-bromophenacyl bromide (1.8 mM) totally prevented the myotoxicity caused by acanmyotoxin-1 (10^{-6} M). In conclusion, myotoxicity of *A. rugosus* venom may be of important clinical relevance given the use of anticholinesterases to reduce the amount of death adder antivenom administered.

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Symposium 7 - Paper 3

PURIFICATION AND CLONING OF PSEUDECHETOXIN THAT TARGETS CYCLIC NUCLEOTIDE-GATED ION CHANNELS

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In 1999, we purified pseudechetoxin (PsTx), the first peptide toxin known to block cyclic nucleotide-gated (CNG) ion channels, from the venom of Australian King Brown Snake (*Pseudechis australis*) (1). Here we report the cloning of the cDNA encoding PsTx from the venom gland of *Pseudechis australis* using RT-PCR. The mature protein is 211 amino acids in length and highly enriched in basic amino acids. The amino acid sequence is 53.1 % identical to helothermine from the Mexican beaded lizard (*Heloderma horridum horridum*), which inhibits voltage-gated calcium and potassium channels, and ryanodine receptors (2), and also homologous to ablomin (60.8%, *Agkistrodon blomhoffi*), triflin (61.5%, *Trimeresurus flavoviridis*), and latisemin (76.8%, *Laticauda semifasciata*), which attenuate depolarization-induced smooth muscle contraction (3). We have also established the non-denaturing method for the purification of PsTx, in addition to previous method using an HPLC system. The venom of *P. australis* was passed over a gel filtration column, and the PsTx-containing fractions were pooled and subjected to cation-exchanged chromatography. Four hundred micrograms of PsTx was obtained from 1 g of lyophilized crude venom. The present results strongly indicate that several snake venoms contain pseudechetoxin/helothermine-like proteins (1, 3).

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Symposium 7 - Paper 4

PURIFICATION AND PARTIAL CHARACTERISATION OF HYALURONIDASE AND FIBRINOGENASE FROM PIT VIPER (*AGKISTRODON HALYS*) VENOM

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Venom from viper and pit viper snakes has been a major source of proteins affecting haemostasis in the mammalian system. Massive haemorrhage coupled with widespread tissue damage are the main causes of fatality in *Agkistrodon halys* bite victims. Two venom components contributing to these have been purified and partially characterised. These are the spreading factor, hyaluronidase and an anti-clotting factor, fibrinogenase.

Hyaluronidase was purified from *A. halys* venom through a two-step purification process using gel filtration followed by cation-exchange chromatography. The enzyme constituted 0.05% (wt) of the total venom. The pure protein was present as a monomer of 67 kDa with a specific activity of 4200 National Formulary Unit (NFU)/mg as determined by a turbidimetric method (1). Its K_m was 55 μg/ml and V_{max} was 3.8 μg hyaluronic acid hydrolysed per min. Preliminary results showed this to be a glycoprotein. The anti-coagulation protein was purified from the same venom using gel filtration followed by anion-exchange chromatography. The reduced form of this protein had a MW of 35 kDa and showed strong anti-coagulant activity in the thrombin time assay using rabbit blood. Further analyses indicated that this protein selectively cleaved the α, β-fibrinogen from both bovine and human sources.

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Symposium 7 - Paper 5

WAPRINS: A NEW FAMILY OF PROTEINS FROM SNAKE VENOMS

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Snake venoms are rich sources of pharmacologically active polypeptides and proteins. These toxins belong to a small number of well-defined protein families. The members in a single family show remarkable similarities in their primary, secondary and tertiary structures. At times, however, they differ from each other in their biological targeting and hence their pharmacological effects. In other words, each family of protein toxins has a similar molecular scaffold but exhibit multiple functions. Some of the well-recognized superfamilies of venom proteins are: (1) phospholipase A_2 (PLA_2) family; (2) serine proteinase family; (3) metalloproteinase family; (4) three-finger toxin family; (5) proteinase inhibitor family; and (6) lectin family. We along with others recently identified helveprins, a new family of toxins. The members of this family show strong homology to helothermine from Mexican lizard venom. Here we describe the purification and amino acid sequence of another new family of toxins. So far, we have identified three members of this family. Because of their structural homology with whey acidic proteins (WAP), we have named them as WAPRINS (WAP related proteins). The functional characteristics and the biological properties of this group of proteins is not known.

Symposium 8 - Paper 1

THE KNOWLEDGE OF NORTH QUEENSLAND ISLAND FERRY PASSENGERS ABOUT IRUKANDJI JELLYFISH

Simone Harrison, Peter Leggat and David Dürrheim, James Cook University; Peter Fenner, Surf Living Saving Association; and Anne Swinbourne, Tropical Public Health Unit

Background: Recent severe Irukandji jellyfish stings in North Queensland have led to calls for increased public awareness of the dangers of these marine creatures. However, little is known about the knowledge of tourists and locals at risk of contact with these jellyfish. A survey was conducted to assist in the development of targeted educational strategies.
Methods: 224 passengers travelling on Sun Ferries to Magnetic Island were approached for interview on 27 April 2002 and 208 (93%) agreed to participate.
Results: Seventy-two percent (n=150) of the passengers claimed to know what an Irukandji was (88.1%) of locals compared to 51.1% of tourists (p=0.00005). Most locals knew that stinger net enclosures would not prevent Irukandji entry, while fewer tourists knew this (p=0.00005). Fifty-eight percent of tourists surveyed were not aware of Irukandji risks before arriving in North Queensland, being 50% of domestic tourists and 63.8% of international tourists. In contrast, most (85.7%) tourists surveyed (93.3% of domestic and 80.9% of international tourists) were familiar with large box jellyfish (*Chironex fleckeri*) risks before arriving.
Conclusions: The majority of respondents claimed to know what Irukandji were. The finding that many tourists, particularly those from abroad, were unaware that stinger nets could not prevent irukandji entry was of concern. Most respondent thought that an irukandji was much larger than it actually is, incorrectly assumed that the Irukandji were confined to North Queensland, and believed that they were safe from Irukandji when swimming on the outer Barrier Reef, especially tourists. Implications for development of education material are raised.

Symposium 8 - Paper 2

IMPORTED PARASITIC SKIN INFECTIONS IN TASMANIA

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In Tasmania, endemic parasitic infections and infestations are usually confined to headlice, pubic lice, scabies, animal mites, Ixodid ticks and leeches. Ophthalmomyiasis due to *Oestrus ovis* has also been recognised.

Imported parasitic skin infections are also being encountered more frequently. Thus infections diagnosed in Tasmania have included: cutanrous leishmaniasis caused by *Leishmania braziliensis* from Brazil cutaneous myiasis due to *Cordylobia anthropophaga* from Malawi and *Dermatobia hominis* from Latin America; *Tunga penetrans* from Africa; cutaneous larva migrans from Brazil, Malaysia and the Pacific Islands and a case of a cutaneous lesion from Zimbabwe, probably due to a dirofilarial nematode.

We have additionally encountered a case of delusional parasitosis in a returned traveller – a condition which needs differentiation from true, authentic infection.

Symposium 8 - Paper 3

MANAGING DEATH ADDER BITE WITH PROLONGED PRESSURE BANDAGING

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Many people bitten by death adders in PNG cannot reach a hospital with a stock of antivenom. There needs to be an alternative management plan for these patients.

Proximal spread of snake venom in lymph vessels can be effectively delayed by the pressure immobilization method. Conventionally, this has only been used as a short term first aid measure. This retrospective study shows that prolonged application of the pressure bandage followed by graded cautious release is effective in managing most cases of death adder bite.

At Rumginae Hospital in the Western Province of Papua New Guinea, in the 5 year period from August 1994 to July 1999 there were 44 admissions for death adder bite with unequivocal signs of envenoming. All were managed with pressure bandaging, strict bed rest and close observation. The graded removal of the bandage was commenced when there were no longer any signs of envenoming, and at least 24 hours after the time of the bite.

Three cases (7%) required antivenom for severe central muscle weakness. There were no deaths. There were no significant local complications from the prolonged application of the pressure bandage.

Prior to the availability of antivenom death adder bite carried a 50% mortality. This study shows that prolonged application of the pressure bandage followed by graded cautious release can be used where there is no antivenom available.

Symposium 8 - Paper 4

THE GREEN PIT VIPER BITE - AN IRISH ADVENTURE

Dagmara Poprawski

The green pit viper is not an Australian native amongst snakes, but can be found in Asian countries, as well as East Timor. While it looks quite pretty, it can be aggressive and bite the unsuspecting victim in woody areas, such as banana plantation.

This is a case presentation of an Irish Peacekeeper in East Timor, who was brought into UN Miolitaray Hospital in Dili with a Snake bite. A positive recognition was made by viewing the snake which was brought in to the Resuscitation Station with the patient.

The case looks at resuscitation measures taken and the options available, including the anti-venin. His follow up care in both High Dependency and the ward. Complications of the bite are discussed and long term outcome of the patient including his return home.

While a rare event, any doctor working in a region where the green pit viper is found must be prepared for the potential of a bite victim coming under their care. The controversy existing in management due to its rarity is important to be remembered so the patient's well being is not compromised.

Symposium 8 - Paper 5

RECENT FATAL BOTULISM IN SOUTH AFRICA

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Although wildfowl and domestic livestock botulism has been recognised as a problem in southern Africa, very few human cases have ever been described in the region. In late February 2002, in Springs, a town on the East Witwatersrand near Johannesburg, two siblings aged 12 and 8 years developed acute flaccid paralysis. One child died before reaching hospital; the other died after 10 days of ventilation in ICU. Mouse bioassays revealed the presence of type A botulinum toxin in the blood of both children, and in the retrieved remains of the implicated food. Botulinum antitoxin for treatment was not available in the country. The implicated vehicle of the toxin was a tin of pilchards (fish of the sardine group) in tomato sauce, commercially produced in South Africa. Type A *Clostridium botulinum* was cultured from the food. The factory production records from the batch of tins showed no apparent deficiencies; likewise post-production testing had been uneventful. Although all unsold tins of the batch were withdrawn, none of these tins have tested positive for botulinum toxin. At this time it appears that the most likely scenario was that corrosion damage had allowed ingress of environmental organisms, including *Clostridium botulinum*, to the tinned food, where suitable conditions for growth and toxin production existed. The investigation into the provenance of the tin, and the circumstances around the consumption of its contents, revealed disturbing lapses in the enforcement of food safety-related legislation by local government health services in Greater Johannesburg.

This is the first outbreak of human type A botulism in southern Africa to be documented, and the first fatal outbreak described; previous human cases in this region have involved type B botulinum toxin, which tends to produce milder disease. A few other outbreaks in elsewhere in Africa have been published, the most extensive being a type E epidemic in Egypt. Commercially tinned products have not been involved in any of these outbreaks.

Symposium 9 - Paper 1

RURAL AND TROPICAL MEDICINE – WHAT'S THAT?

Graeme Schreuder

Chairman, Tasmanian Faculty Board, RACGP. Medical Director, Calvary Healthcare, Tasmania.

The fields of Tropical Medicine, Rural Medicine and Primary Health Care frequently attract a semantic debate as to the nature of these disciplines (1,2). Vocational training is required for all Australian specialties, and medical graduates are cognisant of the need to acquire a Fellowship to gain access to a provider number. However, there are constraints on the terminology used to describe non-mainstream specialties. This paper embraces a multimedia presentation of rural medicine in a tropical environment. Quantitative and qualitative data are presented to illustrate both the semantic problems and skills required when working in this largely unrecognised field (3,4). The data presented are drawn from a prospective study of 3000 consecutive patients in a Pilbara town (3); a study of 50 consecutive patients seen in Alice Springs Hospital's Emergency Department; and qualitative data from Katherine, Nhulunbuy, and tropical Africa (3,5,6).

The data from Alice Springs Hospital are especially apposite, reflecting precisely the casemix seen by practitioners in many tropical Australian communities that are too small to have resident 'specialists'. This paper illustrates that the latter are of little efficacy in areas where generalists of necessity manage the full range of medicine (3,4). An AMWAC[2] report recognised that *"rural health care should be based on generalists who have broadly based competencies, with specialist support as required"* (7). It will be argued that the term 'general practitioner' may not be entirely apt. The isolation of rural hospitals in the tropics is such that few patients are flown to larger centres, and 'on-call' rosters become unworkable without resident generalists. The size of tropical Australia is not always appreciated, with the tropic of Capricorn running near Rockhampton, Alice Springs, and Exmouth; thus, bisecting Australia. Many communities are isolated, poorly serviced, and suffer disproportionately in terms of morbidity (7,8,9). The intention here is to raise awareness, and provoke discussion in areas of policy, curriculum, and workforce. Perhaps, even a review of an unnamed specialty. 'District Surgeon' is an anachronism, but what to call such generalists? The challenge is to resurrect the discipline. Broad based clinical skills followed by training in Tropical Medicine should, in the author's view, be the launching pad for this *"single most important medical workforce issue"* (7).

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Symposium 9 - Paper 2

REALTIME MOLECULAR EPIDEMIOLOGY

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Recent developments in molecular microbiology have led to a spreading network of public health and hospital laboratories with the capacity to perform molecular typing on bacteria and other microbial pathogens. The technology has stabilised in recent years and in some cases has even been automated. Molecular typing techniques are now available that deliver genetic fingerprinting results in less than 24hr from receipt of pure cultures. This speed of laboratory processing allows outbreak investigators to use molecular typing techniques during the initial stage of an outbreak investigation. Recent experience in WA suggests that close collaboration between disease control, environmental health and public health laboratory staff, combined with judicious use of molecular typing methods, can have a lasting impact on the prevalence of specific infectious diseases in the community. The speed of response now possible from suitably equipped molecular epidemiology laboratories, their ability to archive typing data in digital form and their communication of that data to distant centres will enhance the surveillance of key infectious diseases and accelerate the laboratory response to emerging threats.

Symposium 9 - Paper 3

MICROBIOLOGY TEACHING – ITS IMPORTANCE IN TROPICAL AND TRAVEL MEDICINE AND A CONSIDERATION OF PROBLEMS ASSOCIATED WITH ITS TEACHING AT THE UNDERGRADUATE LEVEL TO MEDICAL STUDENTS

Professor J.M. Goldsmid

Discipline of Pathology, University of Tasmania

Courses in Medical/Clinical microbiology comprise the foundation for an understanding of infectious diseases for medical students. A good knowledge of infectious diseases is central to the good practice of both Tropical and Travel Medicine.

There are problems in Australia (and many other developed countries) in relation to the teaching of microbiology to medical students and these are discussed in the light of experience gained at the University of Tasmania, where an optional undergraduate course in Tropical and Travel Medicine has been in operation for about 5 years.

Symposium 10 - Paper 1

BIOLOGICAL AND CHEMICAL DEFENCE: THE THREAT OF BIOLOGICAL AND CHEMICAL WEAPONS

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The biological and chemical warfare capabilities of state and non-state actors continue to grow world-wide, both in sophistication and breadth. More than a dozen nations, including Iraq, Iran, Libya, Syria and North Korea, are either actively pursuing or possess chemical and biological weapons for use against their enemies. There is also a heightened awareness of the utility of such agents by terrorist groups, a possibly deleterious side-effect of an increased awareness by the general public.

This presentation will look at the growing threat of the use of biological and chemical agents by both national programs and non-state actors, the possible agents which might be considered for use, the technical difficulty of manufacturing and using these agents, and the preparations needed to address these threats. Preparations made to prevent, manage and mitigate these threats will form the basis of Australia's protection against biological and chemical weapon attacks in the future.

Symposium 10 - Paper 2

CLINICAL TOXINOLOGY; A GLOBAL AND AUSTRALIAN PERSPECTIVE

J. White

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Clinical toxinology, though still rarely recognised as a specialty in its own right, is increasingly recognised as a relevant area of medical endeavour. In Australia, the advent of courses in clinical toxinology and increasing interest amongst the emergency medicine fraternity have seen an increase in papers on toxinology. It is not yet clear if this translates to better care for envenomed patients. However the rate of envenoming globally and in Australia remains uncertain, but at least globally, is believed to be high. Global estimates for snakebite alone are as high as 2.5 million cases and 125,000 deaths annually. While courses in clinical toxinology with a global perspective are available, resources are few to enable those countries in most need to send doctors for training. More concerning is the global trend towards reduction in antivenom diversity, availability and affordability. New antivenom technologies are in development, but some of the newer "high tech" antivenoms have proved expensive, potentially limiting use. Fab antivenoms, now in routine use, though effective, have demonstrated problems related to rapid clearance, requiring continuous infusions in some cases. The introduction of IgY antivenoms remains uncertain, but developments in Vietnam have indicated a worthwhile potential for this new class of antivenom. Other forms of venom neutralisation, using natural inhibitors, are yet to be demonstrated as having practical application, thus antivenom remains the principle therapeutic device in treatment of envenoming. Development of a "universal" antivenom would allow economies of scale to be applied to current "leading edge" antivenom techniques, which would possibly enable global availability of antivenom for all medically important species of snake. However, even the availability of such an antivenom would not necessarily reduce the human toll from snakebite, if the health professionals required to use antivenom remain unskilled in its use. Therefore, training in diagnosis and management of envenoming remains an important goal in improving outcomes for envenomed patients.

THE PRODUCTION OF *CALLOSELASMA RHODOSTOMA (CR)* ANTIVENOM FROM EGG YOLK OF HENS IMMUNIZED WITH VENOM. IT' S APPLICATION FOR TREATMENT OF SNAKE BITE PATIENTS IN VIET NAM

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INTRODUCTION: Antivenoms (AV) used to treat snake bite victims are normally obtained from horse sera. However, titers are often low, complement-mediated side effects are common and AV production is costly. Avian acquire passive immunity by transferring maternal immunoglobulins from serum to egg yolk. Furthermore,chicken housing is inexpensive and egg collection is non invasive. The aim of this project was to develop suitable *CR* AV from egg yolk of hens immunised with *CR* venom.

METHODS : Groups of 30 hens were immunised on day 0 by the subcutaneous injection of 0.5 LD50 of *CR* venom Antigen (Ag) (4.539 mg venom/bird kg), emulsified in 0.5 ml of Freund' s complete adjuvant. These injections were repeated 9 times on 3 week interval for each with increasing Ag doses. The specific antibodies for components of the *CR* venom started to appear in serum 3 weeks after the first immunisation, reached a plateau at 6 weeks later and remained stable for 26 weeks. Eggs were collected from the laying hens 6 weeks to 26 weeks after the immunisation schedule. Modified water dilution for *CR* IgY AV purification as follows : Separation of yolks. Precipitation of yolk lipoproteins by dilution 1: 5 of egg yolks, pH = 5.2 . Purification of the aqueous extract by ammonium sulphate 42%.

RESULTS : The titer of *CR* IgY AV was 2-fold higher than the corresponding AV titers obtained from horses. The chicken AV has a 4- fold higher effectiveness at a tenth of the costs of horse AV. To date 30 snake bite patients with severe envenoming have been treated with *CR* IgY AV . Results have been excellent : After 24 hs of *CR* IgY AV therapy, the patients were completely recoved, Lee white. PT, PTT, Fibrinogen & platelet count were returned to the normal, no deaths, no amputations of limbs, compared with horse AV therapy were 48 hs & 2% deaths, 3% amputations & 2 deaths. Side effects of *CR* IgY AV therapy were 3% compared with horse AV were 13%.

CONCLUSIONS : *CR* IgY AV therapy from immunised hens proven to be more effective, safer and economical than current horse AVs for the treatment of snake bite victims in Viet Nam.

Symposium 10 - Paper 4

CLINICAL FEATURES OF BROWN SNAKE (*Pseudonaja* species) ENVENOMING AND THE BROWN SNAKE PARADOX

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Brown snake (*Pseudonaja* sp.) envenoming is now the commonest cause of fatality from snakebite in Australia. The critical clinical features of brown snake envenoming are early collapse and early severe coagulopathy.

Early transient "?hypotensive" collapse may occur from 5 to 30 minutes after the bite, often with brief loss of consciousness. There is then usually full recovery until other features of envenoming occur. Initially this distinct entity was considered possibly due to small molecular weight toxins, which are rapidly absorbed to cause "autopharmacological" effects. However the purified prothrombin activator component of brown snake venom has mimicked this response in dog experiments performed by Tibballs et al., with transient myocardial dysfunction associated with microvascular thrombi postulated to account for the transient hypotension seen. Direct myocardial depression by venom components and specific hypotension-inducing proteins may also contribute to the pathogenetic mechanisms for early hypotension and collapse. Early deaths from brown snake envenoming are unusual, but may result from myocardial depression and hypotension occurring in people with already compromised cardiovascular status who don't recover from the usually transient collapse.

The coagulopathy appears more rapidly and is often more severe than that seen in tiger snakes (*Notechis* sp.) and taipans (*Oxyuranus* sp.) - the other major Australasian elapids with procoagulant venom activity. Incoaguable blood with complete fibrinogen depletion is not unusual within 30 minutes of the bite. In addition to bleeding from mucous membranes and venepuncture sites, it is internal bleeding which can be fatal. Most cases of intracranial haemorrhage from Australasian elapid envenoming have been following brown snake bites. Even with severe coagulopathy, the non-specific systemic features of abdominal pain, nausea, vomiting and headache are often mild or even absent.

The presynaptic neurotoxin from *Pseudonaja textilis*, named textilotoxin, has been described as the most potent neurotoxin isolated from a snake venom. With a molecular weight of around 74,000 it has been called structurally the largest and most complex snake venom neurotoxin known. However despite the potency of textilotoxin it is evident from case series that neurotoxicity is uncommon with brown snake envenoming. This is **"the brown snake paradox"** and requires explanation. It is postulated that slow or poor binding or processing of textilotoxin may account for this. Because of the early hypotensive collapse and rapid onset of coagulopathy with severe brown snake envenoming, antivenom is often given early and in large amounts, possibly averting the slower onset neurotoxicity described in cases before antivenom was available. Limited data from immunoassays of venom and venom components in patient sera suggest there is circulating textilotoxin (or its homologue) after brown snake bite.

Symposium 11 - Paper 1

STRUAN K. SUTHERLAND

J. Tibballs

Australian Venom Research Unit, Dept of Pharmacology, The University of Melbourne

Struan Sutherland was the doyen of medical research and in his time was the ultimate authority on the medical management of envenomation in Australia. After graduating in medicine and serving as surgeon lieutenant in the Royal Australian Navy he started work at the Commonwealth Serum Laboratories (now CSL Ltd) in 1966 initially to gain practical experience in immunology. However, in the next year he was appointed foundation head of Immunology Research and stayed 28 years.

CSL had a proud history of production of antivenoms. It had produced antivenoms to the most dangerous snakes, the Red-back Spider, the Paralysis Tick, the Stonefish and the Box-jellyfish. Struan was destined to produce an antivenom against the Sydney Funnel-web Spider which curiously amongst mammals affects only primates. In 1967 after the death of a child, he re-activated research for an antivenom - a task which had defeated others before him. However, it was not until 1980 that an antivenom raised in rabbits, was ready for testing in monkeys. This was a remarkable scientific achievement. Since then, no person has died from the bite of this formidable animal and in many victims the illness following a bite has been dramatically shortened.

Struan developed techniques which made the medical management of envenomation in Australia the best in the World. He was recognised world-wide with his invention of the pressure-immobilisation technique of first-aid. This simple but effective technique revolutionised first-aid for snake bite and of some other types of envenomation. It made redundant the use of tourniquets and other dangerous first-aid manoeuvres. He developed a snake venom detection kit which enabled doctors working at the bedside to ascertain which snake antivenom should be administered.

Struan was a prodigious author, publishing over 300 scientific and medical articles, numerous chapters in books and the standard Australian medical textbook on the management of envenomation, *Australian Animal Toxins*. His work was temporarily halted however when CSL, on privatisation, suspended his work on venoms and antivenoms. He departed amid controversy – arguing courageously that research is an integral part of antivenom production, and should continue. Now, elsewhere around the globe, institutions which manufacture antivenoms are scaling back or withdrawing from the market because although life-saving, their antivenoms are not profitable products.

With the help of Professor Jim Angus at the Department of Pharmacology of the University of Melbourne he founded the Australian Venom Research Unit in 1994 where research into Australia's venomous creatures continues. We have much to learn about venoms. Struan showed the way for many years until his untimely departure in January 2002.

Symposium 11 - Paper 2

CARDIO-TOXICITY IN IRUKANDJI SYNDROME: A PRELIMINARY STUDY OF SUB CLINICAL INJURY.

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Irukandji Syndrome is a relatively common result of cubozoan envenoming in tropical Australia. It accounted for more than 140 presentations to the Department of Emergency Medicine at Cairns Base Hospital between October and May 2002. Severe myocardial dysfunction presenting as pulmonary oedema or cardiogenic shock is a serious but rare complication of Irukandji Syndrome with cases reported or being reported in the literature.

While severe, clinically blatant pulmonary oedema and/or shock are rare, subtle clinical and or radiologic changes consistent with milder degrees of cardio-respiratory abnormality have been noted in some of our patients. An attempt was made to clinically and radiologically assess patients presenting with Irukandji Syndrome to detect evidence of this subtle abnormality. In addition serial cardiac troponin levels were determined and echocardiography preformed if any assessment feature was abnormal. Several patients with no abnormal cardio-respiratory symptoms had transient elevation of cardiac troponin levels and/or transiently abnormal echocardiography. Skin scrapings to recover nematocysts from those envenomed were examined microscopically in an attempt to identify cubozoan species and to determine if any relationship between symptoms and the species responsible for envenoming.

Symposium 11 - Paper 3

"SEVERE IRUKANDJI SYNDROME" THE EPIDEMIOLOGY, MANGEMENT AND NAME CHANGE?

M Little[1], P Pereira[2], J Seymour[3], R Mulcahy[2], P Cullen[2], T Carrette[3]

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Irukandji syndrome is a condition that was first described by Dr Hugo Flecker in Cairns in 1952. However it was a Cairns doctor, Dr Jack Barnes, in 1961 who captured a jellyfish now called *Carukia barnesi,* and demonstrated that this jellyfish caused Irukandji syndrome by stinging himself, his 9 yo son and lifeguard. All three developed Irukandji syndrome. Neither Flecker nor Barnes described cardiac failure associated with Irukandji syndrome.

Since 1987 life threatening cardiac failure has been reported in a small number of patients who develop Irukandji syndrome. We have identified eleven cases, from the literature and cases we have been associated with. In no cases has the *Carukia barnesi* been identified as the offending jellyfish. In one case a 2mm piece of non *Carukia barnesi* tentacle was recovered from a patient. The jellyfish could not be identified.

Only one patient was stung outside North Queensland, and the majority were stung late in the 'jellyfish season'(March/April). Patients presented in significant pain, hypertensive and tachycardic, although the majority became hypotensive requiring inotropic support.

Where reported, most of the patients developed ECG changes and elevation in cardiac enzymes. Five patients required ventilatory support. Patients developed radiological cardiac failure 1.5 hours – 18 hours post sting, with the 67% developing cardiac failure within 10 hours post sting. All patients who had cardiac echocardiography performed demonstrated global hypokinesis that recovered.

In two patients were pulmonary artery catheters were placed, further evidence of a cardiac cause of the cardiac failure was seen, and their condition improved with the use of adrenalin as an inotrope. Catecholamine surge has been suggested as a cause of the Irukandji syndrome symptoms, however in view of the clinical response to adrenalin, we question whether this is the major cause of the cardiac failure.

We believe that these patients have been stung by an, as yet, undiscovered jellyfish, found in Northern Australia, whose toxin acts on the myocardium. As the "Irukandji" jellyfish *Carukia barnesi* has not been identified to be the offending jellyfish, we believe the nomenclature is confusing, and suggest that the term severe Irukandji syndrome should not be used.

Symposium 11 - Paper 4

CORRELATION BETWEEN SEVERITY OF IRUKANDJI SYNDROME AND NEMATOCYST IDENTIFICATION FROM SKIN SCRAPINGS

Truc Huynh[1], Jamie Seymour[2], Teresa Carrette[2], Richard Mulcahy[1], Peter Pereira[1], Paul Cullen[1], Mark Little[3]

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[3] *Sir Charles Gardner Hospital, Australia*

Jellyfish stings in Northern Australia are a significant cause of morbidity and mortality. In Northern Queensland, Box jellyfish (*Chironex fleckeri*) stings are uncommon, whereas Irukandji syndrome is relatively common during the summer period. During the 2001-2002 jellyfish season, we performed skin scrapings in a number of patients who presented to the Cairns Base Hospital emergency department with Irukandji syndrome. These were examined and the offending jellyfish species were identified based on the unique appearance of the nematocysts.

We hypothesise that different jellyfish species produce Irukandji syndrome of different severity. In this study, we categorised Irukandji patients according to severity, based on clinical and biochemical parameters, and correlate these with nematocyst identification from skin scrapings.

Symposium 11 - Paper 5

THE USE OF PRESSURE IMMOBILIZATION BANDAGES IN THE FIRST AID MANAGEMENT OF CUBOZOAN ENVENOMINGS.

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This study aimed to evaluate whether the application of pressure results in additional release of venom from naturally discharged, vinegar soaked nematocysts of the box jellyfish *Chironex fleckeri*. The results show that large quantities of venom are expressed with the application of pressures similar to that applied by compression immobilization bandages. The volume of venom expressed by this pressure was similar to the quantity expressed upon initial natural discharge of the nematocysts. The current recommended practice of applying pressure immobilization bandages to cubozoan stings may worsen the envenomation. As data now exist that show that pressure immobilization bandages may be detrimental to victims envenomed by cubozoans, we suggest that the current practice of the use of pressure immobilization bandages in cubozoan envenomings be discarded until there is direct experimental evidence to support its use.

Symposium 11 - Paper 6

IN VITRO AND *IN VIVO* ANALYSIS OF 'JIMBLE' JELLYFISH (*CARYBDEA RASTONI*) VENOM

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The 'Jimble' [*Carybdea rastoni*] is small, four-tentacled cubozoan jellyfish widespread throughout the Pacific Ocean. Although it usually causes acutely painful stings with local swelling and blistering only, it has also been implicated in some cases of 'Irukandji-like' syndrome. As part of our investigations of Irukandji and Irukandji-like syndromes, we examined the effects of *C. rastoni* venom *in vitro* and *in vivo*. The jellyfish were captured in Gulf St Vincent, South Australia, and soluble crude venom extracted (CVE) from the tentacles of pooled specimens using a simple mortar and pestle approach. The *in vitro* activity of this extract was analysed using rat isolated mesenteric small artery, guinea pig atria and human coronary arteries. The *in vivo* activity of the CVE was examined in anaesthetised, mechanically ventilated piglets. The CVE (0.1-3μg/ml) contracted the mesenteric small artery. This response was unaffected by ω-conotoxin GVIA (1μM), yohimbine (0.1μM), prazosin (0.1μM), guanethidine (10μM) or tetrodotoxin (0.1μM)(n=6). By contrast, the elicited contraction was reduced up to 50%, but not eliminated, by the L-type voltage-operated calcium channel antagonists (L-VOCC) felodipine (30nM), and nicardipine (0.1μM)(n=2). The positive chronotropic and inotropic responses seen in isolated guinea pig left and right atria treated with the CVE (0.1-3μg/ml) were completely unaffected by propranolol (1μM)(n=2). Concentration-contraction relationships were evident in small (<1mm) and large (4mm) diameter human coronary blood vessels (n=2). CVE (3μg/kg) infused intravenously into the piglets over several minutes caused a small increase in heart rate, systemic arterial pressure and pulmonary artery pressures. These changes were moderate at 10μg/kg and marked at 30μg/kg (n=5). However, in contrast to CVE from *Carukia barnesi*[1], peripheral venous blood samples did not demonstrate any significant change in circulating catecholamines at the peak of tachycardia and systemic hypertension. The L-VOCC blocker verapamil (0.1 mg/kg), infused at the peak of these responses, abolished the tachycardia and systemic and pulmonary hypertension. We conclude that although the systemic cardiovascular effects of *C. rastoni* venom appears outwardly similar to that of *C. barnesi*, a cause of the Irukandji syndrome, the action of the former venom appears predominantly post-junctional. Consequently the 'Irukandji-like' syndrome related to jimble stings is not related to hypercatecholaminaemia but is secondary to a direct, Ca^{2+}-dependent action on vascular tissues.

1. Tibballs et al. (2001) *Anaesth Intensive Care* **29**, 552.

Workshop 1 - Paper 1

MECHANISMS OF ENVENOMING IN BOX JELLYFISH; COULD THIS EXPLAIN DELAYED ONSET OF SYMPTOMS IN IRUKANDJI SYNDROME VICTIMS?

Jamie Seymour

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In human envenomings by cubozoans, two types of stings are recognised, type A and type B. Type A has rapid onset of symptoms while there may be a delay of some 20-30 minutes in type B stings. Ecologically, type B stings do not appear to be advantageous for prey capture, suggestion that either the venom has different effects on humans than it does on prey, or that circulation of venom in humans is different than that of the prey.

For cubozoans that cause type A stings, data suggest that their nematocyst shafts are perforated along the length allowing venom to flow from these holes. In contrast, species that cause type B stings have entire shafts, resulting in venom exiting the shaft only at the distal end. It is possible that it is the structural differences in the nematocyst shaft structure between species causing type A and Type B stings that causes the delayed syndromes in victims with type B stings.

When a nematocyst fires, the shaft penetrates the epidermis and may penetrate through several capillaries. Shafts (with perforations) from species causing type A stings would then allow venom to flow directly into the capillaries, analogous to an intravenous injection and give rise to symptoms within minutes. However, shafts (without perforations) from species causing type B stings may pass through capillaries however the probability that the end of the shaft terminating within a capillary would appear small. As such this would be analogous to an intramuscular injection and it would be reasonable to assume that the onset of symptoms may occur approximately 20-30 minutes later.

Workshop 2 - Paper 1

EMERGING INFECTIOUS DISEASES OF THE INDIAN OCEAN RIM (EIDIOR) – THE EIDIOR EVENT MONITOR

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Following its inaugural workshop, the EIDIOR group set up an web-based monitoring service to track infectious disease events in countries bordering on the Indian Ocean Rim. The EIDIOR event monitor presents a simple graphic interface with public domain infectious disease news alerts in the region. This represents new postings on a public access electronic bulletin board. By progressively fading the location indicators, the event monitor provides a visual summary of developing events and a means of instantly assessing their geographical distribution. The simplicity of the event monitor will allow it to expand and develop to meet the interests and needs of centres throughout the IOR region. Rapid growth of the event monitor user group will trigger the next phase of development planned by the EIDIOR steering group: the creation of a mutual capacity-building network, supported by on-line information resources.

157G-2

Workshop 2 - Paper 2

SMALLPOX AND TULARAEMIA: AN UPDATE

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The events of September 11 and the subsequent anthrax letter attacks in the United States have sparked renewed interest in biological agents that could be used as weapons. Smallpox and tularemia are two such agents of concern. This presentation will provide a brief update on these agents, including their potential threat, their clinical syndromes, current treatment and key considerations for their management in the future.

Workshop 2 - Paper 3

Symposium 12 - Paper 1

STRUCTURES OF PHOSPHOLIPASE A_2 GENES EXPRESSED IN SNAKE PANCREAS AND VENOM GLANDS REVEAL THE MOLECULAR EVOLUTION OF TOXIC PHOSPHOLIPASE A_2 GENES

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Three phospholipase A2 (PLA2) genes classified into group IA (two clones) and group IB" (one clone) were isolated from the genomic library of the sea snake *Laticauda semifasciata*. Eight clones of group IA PLA2 were obtained by PCR cloning procedure from genomic DNA of *Laticauda laticaudata* (four clones) and *Laticauda colubrina* (four clones). Comparative analysis of the groups IA and IB" PLA_2 genes revealed that the exon-intron organization is conserved in the genes of both groups (four exons and three introns) like toxic *Naja sputatrix* group IA PLA2 and mammalian pancreas genes. There were two kinds of repetitive sequences in the first (AG rich region) and second (intron II 3' side repetitive region) introns of all sequenced PLA2 genes. The differences in the length of PLA2 genes were derived from the length of their repetitive sequences. Sequences of the repetitive unit of the intron II 3' side repetitive region were highly homologous each other. This observation suggested that the integration of the invaded sequences occurred before the divergence of groups IA and IB" and after the divergence of groups I and II during the evolution of the PLA_2 gene. The chicken repeat-1 (CR1)-like long interspersed repeated DNA (LINE) sequences, different from the above repetitive sequences, were also found in all sequenced *Laticauda* PLA2 genes. A comparative analysis of groups IA, IA' and IIA PLA2s genes suggests a period of CR1-like LINE integration during molecular and family evolution. The integration of CR1-like LINE into PLA2 genes occurred after the divergence of groups I and II PLA2s but before the divergence of groups IA and IB" PLA2s. These integration events occurred before the family divergence of *Naja* and *Laticauda*. The presence of CR1-like LINE and a comparison of intron and exon organization showed that the divergence of *Naja* and *Bungarus* occurred before the divergence of *Laticauda* and *Naja*.

Symposium 12 - Paper 2

COMPOSITION OF RUSSELL'S VIPER (RV) VENOM – AN ANALYSIS OF THE EXPRESSED SEQUENCE TAGS FROM RV VENOM GLAND cDNA LIBRARY

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Russell's viper (RV), *Daboia russelli*, is a venomous snake widely distributed in East, South, and Southeast Asia. Expressed sequence data is an important and rapid tool to characterize the pattern of gene expression of an organism. To study distribution of active genes in the RV venom, a cDNA library was constructed from the *D. russelli siamensis* venom glands from Thailand. A total 135 expressed sequence tags (ESTs) have been generated from size-selected clones at random. Single-pass DNA sequence were determined from each clone and searched for homology in the GENBANK database.

We found that 77 (57.0%) of EST clones had significant match to the entries in the database. Approximately half (39) of these clones were identical or related to toxins from snake venoms. The most prevalent cDNA transcripts in the RV venom gland were phospholipase A2 (13 clones (9.6%) of RV-7 form, and 4 clones (3.0%) of RV-4 form), a bradykinin potentiating peptide – C-type natriuretic peptide (BPP-CNP) precursor homolog (5), RV venom factor X activator heavy chain (3), and RV venom factor V activator (2). The phospholipase A2 clones were identical to the previously reported cDNA from *D. russelli formosensis*. The BPP-CNP homologs from RV were highly homologous to the cDNA from *Agkistrodon blomhoffi*. Nucleotide sequences of factor X and V activators predicted the same proteins previously reported in the database. Several potentially novel toxins identified included ESTs remotely related to atrolysin A (*C. atrox*), lebetin 2 alpha and lebetase Le3 (*M. lebetina*), Batroxobin (*B. atrox)*, and a kinin-releasing and fibrinogen-clotting serine proteinase (*B, jararaca*).

The remaining 58 (43.0%) clones of ESTs were unrelated to any sequences in database to date. Our study was the first study of gene expressed in the Russell's viper. These ESTs data provide a resource to identify the expressed pattern as well as used to determine the toxicity compare to other snake venom.

CHARACTERIZATION OF THE GEOGRAPHIC VARIATIONS IN VENOM PHOSPHOLIPASES A_2 OF TAIWANESE BAMBOO VIPER *(TRIMERESURUS STEJNEGERI)*

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Trimeresurus stejnegeri in Taiwan have been found to show exceptionally high geographic variations in the snake morphology (1) and the venom compositions (2). To investigate the divergence at molecular level, venom samples of the viper were obtained from more than ten localities in Taiwan. The phospholipases A_2 (PLA_2s) were purified from each of the geographic sample by gel filtration and reversed-phase HPLC, and their precise molecular weights were determined by ESI-MS spectrometry. Using the cDNA libraries prepared independently from the venom glands of four specimens from different prefectures (Taipei, Yilan, and Pintong), we cloned and sequenced totally six distinct acidic PLA_2s and five basic PLA_2s. Of them five have been published before (3) and the rest are novel sequences. By matching the molecular masses and the N-terminal sequences of the purified PLA_2s to those deduced from sequences of the cDNA clones, the complete amino acid sequences of all the PLA_2s in the venom samples were solved. In general, each of the samples contain 1~3 distinct acidic PLA_2s, a Lys-49 myotoxic PLA_2 and a basic anti-coagulating PLA_2. The acidic PLA_2s share more than 78 % sequence identities and may be classified into 2 subtypes by a phylogenetic analysis. A fast venom evolution under natural selection and multiple migrations of this viper from Mainland China (which also show great geographic variations of the venom acidic PLA_2s) are possible causes of the complex PLA_2-variations observed. The specificities of the acidic PLA_2s toward platelets from different mammals were also tested. The results help to understand the evolutionary adaptation of the viper to the environmental differences.

1. Castellano, S., Malhotra, A. and Thorpe, R.S.(1994)*Biol. J. Linn. Soc.* **52**,365-375.
2. Creer, S. (1999) *Toxicon* **37**, 289-290.
3. Nakai, M., et al. (1995) *Toxicon* **33**, 1469-1477.

Symposium 12 - Paper 4

DEVELOPMENT OF ELISA KITS FOR SPECIES IDENTIFICATION OF VENOMS FROM FOUR SNAKES OF MEDICAL IMPORTANCE IN VIETNAM

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An avidin-biotin ELISA kit was developed for species identification of venoms from four medically important snakes in South Vietnam: Green pit viper (*Trimeresorus popeorum*), Malayan pit viper (*Calloreselasma rhodostoma*), Common cobra (*Naja naja*) and King cobra (*Ophiophagus hannah*). Species-specific antivenom antibodies were prepared by the method based on a three-step affinity purification of the specific antibodies. In the first step, IgG antibody fractions from hyper-immunized rabbits were extracted by affinity chromatography with protein A columns. Next, antivenom antibodies were purified by immuno-affinity chromatography with respective homologous venom columns. In the third step, species-specific antibodies were prepared by immuno-absorbent method in which monovalent antivenom antibody against one snake was adsorbed in sequence onto three venom columns containing other three heterologous venoms for the removal of cross-species reacting antibody molecules. Species-specific antibodies were then used for construction of test kit for the identification of venoms in various types of sample including whole blood, plasma, serum, urine, or sample buffer. The kit can differentiate the venom from these four above snake venoms with the sensitivity at nanogram level within 30 min. The efficacy of the kit for snake venom detection was demonstrated by experimental envenomation of the four snake venoms in rats. Preliminary results with serum samples taken from human snake bite victims in Vietnam show that the kit is applicable for fast identification of snake bites. Larger trial in the field is being carried out.

ELECTROSPRAY LC-MS FINGERPRINTING OF *ACANTHOPHIS* (DEATH ADDER) VENOMS: TAXONOMIC AND TOXINOLOGICAL IMPLICATIONS

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Death adders (genus *Acanthophis*) are unique among elapid snakes in both morphology and venom composition. Despite this genus being among the most divergent of all elapids, the venom has been historically regarded as relatively quite simple. In this study, LC-MS analysis has revealed a much greater diversity in venom composition, including the presence of molecules of novel molecular weights which may represent new classes of venom components. Furthermore, there exists significant variation between species and populations, which allow for the LC-MS fingerprinting of each species. Mass profiling of *Acanthophis* venoms clearly demonstrates the effectiveness of this technique which underpins fundamental studies ranging from chemotaxonomy to drug design.

CARDIOVASCULAR AND HAEMATOLOGICAL EFFECTS OF PAPUA NEW GUINEA SMALL-EYED SNAKE (*Micropechis ikaheka*) VENOM AND THEIR NEUTRALISATION WITH CSL POLYVALENT SNAKE ANTIVENOM

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The Small-eyed Snake (*Micropechis ikaheka*) causes human fatalities in Papua New Guinea, but the actions of its venom are poorly described, and treatment is empirical. Anecdotal clinical effects include paralysis, coagulopathy, myotoxicity and, possibly, haemolysis[1]. This species is part of the Australo-papuan clade of elapids but its taxonomical affinity with Australian elapids is unresolved, hence an appropriate antivenom cannot be predicted from genetic relationships.

We examined the cardiovascular and haematological effects of venom in mechanically ventilated anaesthetised piglets. Freeze dried venom was resuspended in saline with 0.1% bovine serum albumin and intravenously infused in doses 1 - 2000 μg/kg. Reactivity of venom in the CSL Ltd snake venom detection kit (SVDK) was examined for possible antivenom requirements and we examined the effectiveness of CSL polyvalent snake antivenom against toxicity. With high doses (>400 μg/kg), severe pulmonary hypertension, depression of cardiac output and systemic hypotension resulted in death. Significant elevation of pulmonary blood pressure (P=0.002) and depression of cardiac output (P=0.02) were observed for approximately an hour after envenomation with lower venom doses (150 μg/kg) and the concentration of plasma free haemoglobin rose more than 50-fold. No disturbances occurred in routine clinical coagulation tests nor was thrombocytopaenia evident at any venom dose. Creatine phosphokinase levels did not increase after all doses of venom. Cardiovascular effects of venom were absent and haemolysis was decreased when venom was pre-incubated [37°C for 30 minutes] with CSL Ltd polyvalent antivenom but not with Black Snake antivenom before infusion. A concentration response curve for optical density was determined for the SVDK using between 0.01μg-10mg/ml of venom. Immunoreactivity with the Tiger Snake well was visually positive at the lowest venom concentration but remained only weakly positive through to higher venom concentrations. By contrast, the Black Snake well became strongly positive only at very high venom concentrations.

We conclude *M. ikaheka* venom causes severe cardiovascular effects and haemolysis. Cardiovascular effects were neutralised and haemolysis was reduced by CSL polyvalent snake antivenom.

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Symposium 13 - Paper 1

CHRONIC ENVENOMATION SYNDROMES

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Follow-up of victims who have been subjected to a variety of marine and terrestrial envenomations, has shown that many victims require attention over a prolonged period of convalescence. Months rather than weeks is the usual time for recovery after significant envenomations; and for some types of clinical poisoning, such as ciguatera, the convalescent period may stretch to years rather than months.

Acute envenomation following jellyfish stings, fish spine envenomations, snake bite and platypus envenomations may be prolonged. Although initial symptoms can be rapidly reversed, when appropriate antivenom is available, some damage which occurs prior to antivenom reversal, requires the regeneration of new tissue, particularly excitable tissue such as muscle. Local injected venoms may leave pigmentation, fat atrophy, mononeuritis and foreign-body granulomas which may persist for months or longer. This paper highlights the importance of the persistence of chronic post-envenomation symptoms, even after optimal acute management.

Symposium 13 - Paper 2

DEATHS FROM SNAKEBITE IN AUSTRALIA 1979-2000

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Aims:
To characterise venomous snakebite mortality in Australia from 1979 to 2000. To examine trends in mortality over time. To identify potential risk factors and develop prevention and primary management strategies aimed at reducing mortality from venomous snakebites in Australia.

Methods:
A search for venomous snake bite related fatalities that occurred during the period January 1979-December 2000 was made using the Australian Bureau of Statistics mortality dataset, State and Territory Registrars of Births, Deaths and Marriages and coronial authorities. Identified fatalities were investigated utilising the case files of these agencies and, where necessary, the relevant hospitals and medical practitioners.
The project was approved by the University of Melbourne Health Sciences human ethics subcommittee.

Results:
Fifty-eight deaths (42 males and 16 females) were reported in relation to venomous snakebite between January 1979 and December 2000. Most deaths (21 = 36%) occurred in Queensland, and most (>85%) fatal snakebites occurred in rural areas. Brown snakes were identified in 22 cases (38%), and tiger snakes in 11 cases (19%). Early collapse or out of hospital cardiorespiratory arrest occurred in 29% of cases, including 64% of brown snake fatalities and 55% of tiger snake fatalities.

Discussion:
Death from venomous snakebite in Australia is relatively uncommon, probably due to low exposure of the highly urbanised population to venomous snakes, the availability of high quality antivenoms and ready access to medical facilities, including intensive care services. Despite advances in medical care and emergency transport, mortality rates have not significantly decreased over the 22 year study period. This is probably related to the high proportion of out of hospital cardiorespiratory arrests in snakebite victims. Snakebite mortality might therefore be reduced by prevention of snake bites and by the prompt use of effective first aid measures. Education on prevention and first aid, particularly in at risk rural communities, may be beneficial.

Symposium 13 - Paper 3

BROWN SNAKE ENVENOMING IN WESTERN AUSTRALIA ARE WE GIVING ENOUGH ANTIVENOM?

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The Australian Brown snake venom (*Pseudonaja* species) has strong prothrombin (Factor Xa like) activator activity. Envenomation often results in a defibrinating coaguloapthy.

Invitro[1] work in dog models demonstrates that a larger dose (10 – 20 times the expected dose) of Commonwealth Serum Laboratories (CSL) brown snake antivenom prevents the procoagulant action of Brown snake venom.

CSL currently recommends an initial dose of 4 ampoules of antivenom for a major coagulopathy associated with a Brown snake envenoming

As major haemorrhage is a rare but potentially life threatening condition we believe it is important to reverse the envenomation and coagulopathy as soon as practical.
We present a retrospective review of cases of Brown snake envenoming seen in Western Australia. Based on this data, and that of animal work, it appears that the current CSL antivenom recommendations are insufficient for patients envenomed by brown snakes in Western Australia.

We will present our current recommendations for brown snake antivenom dosing.

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Symposium 13 - Paper 4

BIODISTRIBUTION OF INTRAMUSCULAR AND INTRAVENOUS INJECTION OF F(ab')$_2$ ANTIVENOM

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We studied the biodistributions of F(ab')$_2$ anti-Malayan Pit Viper venom (AV) given intramuscularly (IM) compared to intravenously (IV) in normal mice and mice with turpentine-induced inflamed muscle. Four groups of mice were given Tc99m-labelled purified equine F(ab')$_2$ AV, i.e. group I-normal mice with IV injection, group II-inflamed mice with IV injection, group III-inflamed mice at right femur with contralateral IM injection, and group IV-inflamed mice with IM around the inflammation. Mice were sacrificed at 1, 4, or 24 hours after injection and radioactivity (as % of injected dose per gram tissue, %ID/g) was determined in each dissected organ: liver, spleen, kidneys, lungs, heart, stomach, intestine, bone, blood(cardiac), and injected muscle, inflamed or normal muscle.

We found that when AV was given IV (group I, II), radioactive F(ab')$_2$ AV was distributed mostly in kidneys (mean ± SD = 46 ± 29 %ID/g, n =5) at 1 hour after injection. It then decreased to 34 ± 3 and 17 ± 6 %ID/g at 4 and 24 hours respectively, while AV were <2 %ID/g in all other tissues. Inflamed muscle did not have significantly higher uptakes of AV than normal muscular tissue. Intramuscular injection (group III) results in slightly lower peak level of distribution of AV to the kidneys (34 ± 12 %ID/g) at 1 hour and sustained (34 ± 31 %ID/g) at 4 hours then decreased to 17 ± 8 %ID/g at 24 hours. Only a small amounts of AV remained at the injection site (3.6 ± 2.9 %ID/g) at 1 hour and decreased further to 0.9 ± 0.6 and 0.4 ± 0.3 %ID/g at 4 and 24 hours respectively. This indicates that F(ab')$_2$ AV is rapidly diffused from the injection site. When injected around the inflamed muscle (group IV), the distribution pattern is similar to group III mice, except that AV is retained at the injected inflamed muscle at higher level (5.7 ± 1.1, 3.3 ± 1.4 and 1.3 ± 0.6 %ID/g at 1, 4 and 24 hours respectively), than in normal muscle.

We concluded that F(ab')$_2$ AV effectively diffuses from an intramuscular injection site, concentrates in the kidneys from where it is probably eliminated. Our data suggests that intramuscular administration of F(ab')$_2$ AV may be an alternative to the intravenous route because of easier technique. Further animal experiments are needed to elucidate whether intramuscular administration of F(ab')$_2$ antivenom may have clinical applicability.

Symposium 13 - Paper 5

ORAL PREDNISONE FOR LIMB EDEMA IN CHILDREN WITH GREEN PIT VIPER BITES: A RANDOMIZED CONTROLLED TRIAL

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Green pit viper (GPV) bite is the most common snake bite in Bangkok and vicinity. *Trimeresurus albolabris* and *T. macrops* are responsible for most cases of GPV bites, which result in mild coagulopathy, but marked limb edema without extensive necrosis. Steroid is a broad spectrum anti-inflammatory agent. We previously investigated whether low-dose oral prednisolone and/or amoxicillin is useful for treatment of GPV bite in children. The result of an open label-randomized trial suggested that prednisolone at 1 mg/kg/day given with amoxicillin may reduce edema more rapidly than amoxicillin alone (1).

To confirm these findings, we conducted a double-blind randomized placebo-controlled trial comparing oral prednisolone at 1 mg/kg/day and placebo for 3 days and measure limb circumference daily for 3 days. Antibiotics were not given. Only children who were bitten on a limb within 24 hours, and did not receive any first aid treatment were eligible for the study. Chidren with severe coagulopathy necessitating antivenom treatment were excluded from the study prior to randomization. Forty-three children (mean age 7.8 $\pm$ 4.0 years) with GPV bites were included in this study. Twenty-two were randomized to receive prednisolone and 21 receive placebo. The age and sex distribution, transit time to hospital, coagulation parameters at presentation, and limb circumference at presentation were comparable between the two groups.

Edema of the bitten limb, measured by circumference at the mid-palm, mid-sole, or across the bitten site, were evident (1.8 $\pm$ 1.0 cm greater than the contralateral limb) on the initial visit (day 0), which increased to 1.9 $\pm$ 1.2 cm around 24 hour after bite (day 1), then decreased to 1.5 $\pm$ 1.0 cm and 1.1$\pm$ 1.0 cm at 48 hours (day 2) and 72 hours (day 3) respectively. The extent of limb edema did not significantly differ between the prednisolone group (average circumference increase = 1.9, 2.2, 1.6 and 1.1 cm on day 0, 1, 2, and 3 respectively) and the placebo group (1.6 cm, 1.7 cm, 1.4 cm, and 1.1 cm on day 0, 1, 2, and 3 respectively). A subgroup analysis comparing only moderate (greater than 1.5 cm) or mild edema (<1.5 cm) did not show that steroid is better than placebo in reducing limb edema.

In conclusion, we could not demonstrate the benefit of low-dose steroid in reducing limb edema from green pit viper bite in children. Although pathogenesis of limb edema in green pit viper bite remains unclear, it is probably related to the direct toxic effects of the venom than secondary to mediators of inflammations.

BITES BY SPIDERS OF THE FAMILY THERIDIIDAE : A PROSPECTIVE STUDY OF SPIDER ENVENOMATION.

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Spiders of the family Theridiidae have a world-wide distribution and include at least two medically significant genera, *Latrodectus* spp.(widow spiders) and *Steatoda* spp. (cupboard spiders). It is thought that most *Latrodectus* spp. bites are minor, and uncommonly cause significant envenomation or latrodectism. Despite bites occurring commonly around the world, there have been no well-designed studies describing the time course and outcome of bites by this family. The study aimed to describe the clinical effects of theridiid spider-bite.

Subjects were recruited prospectively from February 1999 to April 2002 from patients presenting to participating hospitals or contacting a state Poisons Information Centre. 99 cases of bites by spiders of the family Theridiidae were included with a definite bite and collected spider that was identified by an expert arachnologist. Of the 99, 58 were by *L. hasselti*, 21 by *Steatoda* spp., 4 by *Achaearanea* spp. and 16 by undifferentiated theridiid spiders. The seasonal incidence of bites differed, with *Steatoda* bites occurring throughout the year, and *L. hasselti* having a large peak over summer. The major symptom of bites was pain (severe and persistent). The mean duration of pain and standard deviation [SD] of each was *L.* hasselti, 35hr (SD 28hr), *Steatoda* spp., 11hr (SD 12hr) and *Achaearanea* spp., 16hr (SD 11hr), with a significant difference between the first two ($p<0.0001$). 66% of *L. hasselti* bites caused moderate/severe pain lasting over 24 hours. Only *Latrodectus* bites caused sweating (31%), but systemic effects occurred in 33% of *Steatoda* bites which was not different to 36% of *L. hasselti* bites ($p=1.00$). 26 *L. hasselti* bites were seen in hospital, 8 of these treated with intramuscular antivenom. There was no significant difference in pain duration ($p=0.63$) with untreated patients.

The study shows that bites by spiders from the family Theridiidae cause similar effects, but most severe with *L. hasselti*. It also shows that the seriousness of latrodectism in Australia has been underestimated, with a majority of cases having severe pain for over 24 hours. Treatment practices for latrodectism need review and patients should be offered appropriate antivenom treatment. The study confirms the isolated clinical cases of *Steatoda* bites and *in vitro* data that *Steatoda* causes similar but less severe effects than *Latrodectus* [1]. This is the first report of bites by *Achaearanea* spp. and it demonstrates that another theridiid genus causes similar clinical effects.

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Symposium 14 - Paper 1

PHARMACOLOGY AND SAFETY OF THERAPEUTIC BOTULINUM NEUROTOXIN PREPARATIONS

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The bacterial neurotoxin purified from clostridium botulinum type A has been approved and successfully utilized in the treatment of various medical conditions since 1989 in the United States, thus fulfilling Justinus Kerner's 1822 prediction of the therapeutic value of the "fatty substance from sausage". Since the original approval, two other products have been approved in 1991 (another serotype A first approved in the United Kingdom) and 2000 (serotype B, first approved in the United States). The biological activity of these preparations is described as mouse lethality units, as defined by each manufacturer. However, due to the differences in bulk material, formulations and MLU definitions, the Units (U) are neither equivalent nor interchangeable. This presentation will review the history, development and pharmacology/safety of the commercial preparations containing botulinum neurotoxin.

Symposium 14 - Paper 2

ROLE OF ALPHA-TOXIN AND PERFRINGOLYSIN O IN *Clostridium perfringens*-MEDIATED GAS GANGRENE

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The anaerobic bacterium *Clostridium perfringens* is a normal inhabitant of the gastrointestinal tract and is the causative agent of gas gangrene or clostridial myonecrosis. An unusual feature of this often fatal disease is that no leukocyte accumulation is observed in the infected tissues. *C. perfringens* produces more than ten different extracellular toxins and enzymes, only two of which have been shown to be involved in the pathogenesis of gas gangrene. The alpha-toxin has both phospholipase C and sphingomyelinase activity and is an essential toxin in the disease process. Alpha-toxin mutants are avirulent in a mouse myonecrosis model, with comparative histological examination of muscle tissue from mice infected with a series of isogenic strains indicating that this toxin is required for tissue necrosis, inhibition of the influx of polymorphonuclear leukocytes into the lesion, and thrombosis formation. Perfringolysin O or theta-toxin is a cholesterol-dependent cytolysin that has been implicated in the vascular accumulation of leukocytes within blood vessels and the extracellular matrix of the host tissues. Although it therefore has the ability to affect the host inflammatory response, the toxin is not essential for virulence since perfringolysin O mutants still cause disease. By insertional inactivation we have constructed a chromosomal *C. perfringens* mutant that is unable to produce either alpha-toxin or perfringolysin O and therefore is avirulent in the mouse model. This mutant was complemented with separate recombinant plasmids that carried the structural genes for either alpha-toxin or perfringolysin O, or carried both toxin genes. Virulence was restored when the mutant was complemented with the alpha-toxin structural gene, but reconstituting only perfringolysin O activity produced vastly different results, with regions of coagulative necrosis, apparently enhanced by vascular disruption, being observed. Reconstitution of both alpha-toxin and perfringolysin O activity produced a histopathology most similar to that observed with the alpha-toxin reconstituted strain. The results clearly support the hypothesis that alpha-toxin is the major toxin implicated in the disease but indicate that perfringolysin O does have a synergistic role in the pathology of the disease.

Symposium 14 - Paper 3

STRUCTURAL STUDIES OF PORE-FORMING PROTEIN TOXINS

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Despite the fundamental and fascinating functions that ion channels fulfil, there are still no three-dimensional structures available of an eukaryotic ion channel. There are a number of reasons for this anomaly: the natural abundance of ion channels is low, expression levels are often poor, they can be difficult to purify and even more difficult to crystallize. We have developed an alternative approach that makes use of the observation that many bacterial protein toxins kill cells by forming voltage-gated channels. We have thus pursued structural studies of pore-forming toxins as model systems for understanding the biogenesis, structure and function of membrane ion channels. We have determined the crystal structures of a number of microbial pore-forming toxins: colicins (1,2), aerolysin (3,4), perfringolysin O (5-7) and gamma-hemolysin. Although the structures are quite different they reveal common features that have been implicated in the mechanism of membrane insertion.

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Symposium 14 - Paper 4

SYNTHETIC GLYCOSYLPHOSPHATIDYLINOSITOL AS A CANDIDATE ANTI-TOXIC VACCINE AGAINST MALARIA.

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Plasmodium falciparum malaria infects 5-10% of humanity and kills two million people annually. Fatalities are thought to result in part from an inflammatory cascade initiated by an endotoxin-like molecule of malarial origin. Glycosylphosphatidylinositol (GPI) of *P. falciparum* origin has the properties predicted of a toxin, but a requirement for toxins in general and GPI in particular in malarial pathogenesis and fatality remains unproven. As anti-toxic vaccines can be highly effective public health tools, we sought to determine whether anti-GPI vaccination could prevent pathology and fatalities in a murine model of severe malaria, with similarities to the human condition in inflammatory aetiology and metabolic derangement. The *P. falciparum* GPI glycan of the sequence NH_2-CH_2-CH_2-PO_4-(Manα1-2)6Manα1-2Manα1-6Manα1-4Glc$NH_2\alpha$1-6*myo*-Inositol-1,2-cyclic-phosphate was chemically synthesized, conjugated to protein carriers and used to immunized mice. Recipients were substantially protected against severe pathology, acidosis, pulmonary oedema, cerebral syndrome and fatality. Anti-GPI antibodies also neutralized pro-inflammatory activity by *P. falciparum in vitro*. GPI is thus a significant pro-inflammatory toxin of parasite origin, and multiple disease parameters in malaria-infected mice are toxin-dependent. Synthetic GPI is therefore a candidate carbohydrate anti-toxic vaccine against malaria.

Workshop 3 - Paper 1

A NOVEL BROWN SNAKE *PSEUDONAJA TEXTILIS* AVIAN IgY AND OVINE IgG ANTIVENOM WITH IMPROVED EFFICACY AGAINST THE MAJOR TOXIC ACTIVITIES.

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Brown snakes, *Pseudonaja* genus, cause the most snakebites and deaths to humans and animals in Australia. Over recent years, it has been shown both experimentally and clinically, that reversal of the prothrombin activator in this venom is difficult using existing antivenoms. A new brown snake antivenom has been developed which promises to solve the problem of neutralising this component. We present evidence here, which demonstrates that this newly developed antivenom (Antiven Pty Ltd Brown snake antivenom) is superior at neutralising the procoagulant and neurotoxins and shows considerable improvement in the venom neutralisation kinetics.

Workshop 3 - Paper 2

ARE POLYSPECIFIC ANTIVENOMS REALLY POLYSPECIFIC?

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Polyspecific antivenoms are raised on the basis of venoms from multiple species of venomous organisms. The inclusion of venom of a given species in the manufacture of the antivenom is normally taken as an indication that the antivenom will be effective in neutralising the venom of any population of that species. Here, we test this assumption in South American pitvipers of the *Bothrops atrox* species complex. The mouse ED_{50} and MHD Neutralising Doses of a broad-spectrum, anti-*Bothrops* polyspecific antivenom (Sôro Antibotrópico, Instituto Butantan) were assayed for eleven regional venom pools of the *B. atrox* complex. All venoms from populations included in the manufacture of the antivenom were effectively neutralised. However, the venoms from other populations varied hugely in the extent to which their lethality or haemorrhagic activity were neutralised by the antivenom. The effectiveness of the antivenom was not predicted by either the phylogenetic position or the species affinities of the populations.

Obviously, the results of mouse tests may not necessarily predict clinical results in humans. Nevertheless, these results show that even polyspecific antivenoms may potentially fail to neutralise at least some activities of venoms of some populations of species included in their manufacture. In view of the ubiquity of intraspecific venom variation in snakes, and the fact that venom often varies independently of species affinities and phylogeny, this may be an important consideration in the manufacture of antivenoms. We recommend the following: (i) Antivenoms should be produced against the species and populations responsible for the majority of bites, wherever these may occur, and not against similar species or conspecific populations that happen to be conveniently available, but are not in fact responsible for many bites; (ii) Antivenoms should be raised from venoms collected across the entire range of each species across the target region. This concern is particularly applicable to venom producers who rely on captive-bred stocks of venomous snakes: often, these stocks originate from a few specimens from a single locality, and probably contain only a fraction of the total spectrum of antigens present in the species as a whole; (iii) At the very least, antivenoms should be tested for neutralising ability against the venoms of all major populations of any species, with special emphasis on those occurring in regions with a high incidence of snakebite.

Symposium 15 - Paper 1

ADDING VALUE TO MARINE TOXINS

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Studies on marine natural products over the past 50 years have led to the discovery of many novel structures, some with such potent biological properties that they are best described as toxins. These compounds are not toxins in the conventional sense of the word – shellfish toxins or peptide toxins – but they are nonetheless compounds with comparable potencies. Unlike many of the conventional toxins, these bioactive compounds are not normally ion-channel active. Rather, they act to inhibit processes such as mitosis.

In work in our laboratories in New Zealand we have isolated many such toxins over the past two decades. The chemistry of selected samples such as the halichondrins, mycalamides and variolins will be described but the emphasis will be less on the compounds themselves and rather more on how the effectiveness and value of these compounds can be enhanced by conversion to **polymer therapeutics**. Polymer therapeutics is a relatively new approach to drug delivery that leads to passive, as well as active targeting of the biological target, a defined method of release of the drug, and overall superior pharmacokinetics.

At Canterbury we are actively working in both the cancer and AIDS area to establish proof-of-principle of this approach to drug delivery using marine-derived toxins. Results from this approach will be presented.

Symposium 15 - Paper 2

DISCOVERY, STRUCTURES AND INSECTICIDAL PROPERTIES OF THE CYCLOTIDES: CIRCULAR MINI-PROTEINS FROM PLANTS

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The cyclotides [1] are a recently discovered family of small plant-derived proteins that have a range of exciting applications in drug design and agriculture [2]. They are typically about 30 amino acid in size, contain a head-to-tail cyclised backbone and incorporate three disulfide bonds arranged in a cystine knot topology. In this motif an embedded ring in the structure formed by two disulfide bonds and their connecting backbone segment is penetrated by the third disulfide bond. The combination of this knotted and strongly braced structure with a circular backbone renders the cyclotides impervious to enzymatic breakdown and makes them exceptionally stable. Their stability is shown to be critically dependent on the integrity of the cyclic cystine knot (CCK) motif, as demonstrated by the synthetic production of acyclic permutants in which the backbone is broken at locations that formally remove the knotted topology. The cyclotides are gene products derived from linear precursor proteins that encode either one, two or three cyclotide domains. The cyclotides are the first example of a growing number of naturally occurring circular proteins discovered over recent years [3] that appear to play a crucial role as host defence molecules. This presentation will describe the discovery of the cyclotides in plants, their three-dimensional structural characterization by NMR and their potential application as insecticidal agents [4]. We have also undertaken extensive studies on the folding mechanisms of the cyclotides. The native peptides were reduced and then monitored by HPLC and NMR during re-folding and a stable two-disulfide intermediate has been isolated. This has provided an insight into the mechanism of formation of the cyclic cystine knot motif.

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Symposium 15 - Paper 3

ShK Toxin as a Potential Diagnostic Tool for Relapsing-Remitting Multiple Sclerosis

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Multiple sclerosis (MS), a chronic autoimmune disease, is characterized by inflammation of the central nervous system resulting in severe neurological impairment and eventually death of the affected patients. Myelin reactive T lymphocytes have been implicated in the pathogenesis of MS due to their ability to induce experimental autoimmune encephalomyelitis (EAE) following adoptive transfer in animal models. Selective suppression of myelin-reactive T cells therefore represents a potential therapy for the treatment of MS.

We have investigated the effects of ShK toxin, a potent blocker of the lymphocyte potassium channel Kv1.3, on EAE induced by an MBP-specific T cell line (TCL) in rats. Administration of this peptide toxin, which is isolated from the sea anemone *Stichodactyla helianthus*, could both prevent and treat the symptoms of EAE (1). In contrast to normal mitogen activated T cells, which rely on a different potassium channel for their activation, these disease inducing MBP-specific T cells express very high numbers of Kv1.3 channels and their proliferation is extremely sensitive to Kv1.3 blockade.

More recently, we have initiated studies on T cells isolated from MS patients to determine if they exhibit the same $Kv1.3^{high}$ phenotype. Upon stimulation of T cells from MS patients with myelin basic protein, these MBP-specific T cells expressed dramatically higher numbers Kv1.3 relative to control TCLs from healthy volunteers suggesting that they have undergone repeated antigenic stimulation *in vivo* during the course of the disease. Using flow cytometric studies, the T cell subset expressing this $Kv1.3^{high}$ phenotype were determined to be effector memory cells due to the lack of both CCR7 and CD45RA which are T cell surface markers indicative of naïve and central memory cells.

Our current work focuses on developing a fluorescein labeled derivative of ShK toxin for flow cytometry as a diagostic probe for $Kv1.3^{high}$ myelin specific cells in MS. Flow cytometry is feasible because high numbers of Kv1.3 in activated MBP-specific effector memory T cells are within the detection limits of this technique. Thus, we prepared an N-terminally fluorescein-6-carboxyl-ShK (ShK-F), which maintained low picomolar affinity for the channel. As ShK binds to the extracellular vestibule of Kv1.3, no cell permeabilization was required. We suggest that using ShK to specifically target activated myelin-reactive $Kv1.3^{high}$ effector memory T cells could constitute a new therapy for MS, while ShK-F could serve as a new diagnostic marker for the detection of activated effector memory T cells.

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Symposium 15 - Paper 4

DEADLY SPIDER MAKES GOOD—DISCOVERY, PHARMACOPHORE MAPPING, AND COMMERCIAL APPLICATIONS OF INSECTICIDAL NEUROTOXINS FROM AUSTRALIAN FUNNEL-WEB SPIDERS

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In addition to destroying an estimated 20–30% of the world's food supply, arthropod pests are responsible for the transmission of many new and reemerging human diseases. These arthropods have largely been controlled by spraying broad-spectrum chemical insecticides. However, the long-term application of a small armament of insecticides that act on a restricted number of invertebrate nervous system targets has inevitably led to the development of resistance in most agrochemically and medically important arthropods. The emergence of insecticide-resistant insect populations, as well as increasing disquiet about the environmental and human health risks associated with certain agrochemicals, has stimulated the search for new arthropod-control strategies.

Since the primary role of spider venoms is to kill or immobilize arthropod prey, it is not surprising that these venoms have proved to be rich sources of insecticidal compounds. By systematically screening the venom of the lethal funnel-web spider *Hadronyche versuta* we have discovered several families of novel insect-specific toxins (1–3). These toxins are especially valuable from the viewpoint of insecticide design because they act on, and have enabled us to validate, nonconventional neuronal targets. Furthermore, alanine scanning mutagenesis has revealed that the pharmacophore of each toxin is restricted to a small number of spatially contiguous residues, thus increasing the probability of designing functional small-molecule analogs (4,5).

Although all of these toxins are members of the inhibitor cystine-knot family, they have proved to be structural chameleons, with the 3D fold generally providing few clues about toxin function. Thus, in order to delineate the exact molecular targets of these toxins, we have developed an unbiased genetic screen using transgenic flies. We will describe work on two lines of flies that carry transgenes for funnel-web spider toxins.

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Symposium 16 - Paper 1

TARANTULA TOXINS AS DISCOVERY TOOLS FOR THE STUDY OF ACID-SENSING AND VOLTAGE-DEPENDENT CATIONIC CHANNELS

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Spider venoms are a unique source of toxins for Kv2.1, Kv4.2 and Kv4.3 potassium channels. Most toxins discovered to date are short peptides, highly reticulated by disulfide bridges which belong to the same structural family of Inhibitor Cysteine Knot (ICK) peptides. They act by modifying the kinetics of either inactivation or activation gating through interaction with the voltage-sensing domain of Kv channels. We have identified several novel tarantula toxins from the venom of the African tarantulas *Stromatopelma calceata* (ScTx1) and *Heteroscodra maculata* (HmTx1, HmTx2). These peptides are potent blockers of Kv2 and Kv4 channel subtypes. ScTx1 is a high affinity blocker for both I_A and I_K currents and is the first described high-affinity inhibitor of the Kv2.2 channel subtype (IC_{50} 21.4 nM). It also blocks Kv2.1 channels (IC_{50} 12.7 nM) as well as Kv2.1/Kv9.3 heteromultimers that have been proposed to be involved in O_2 sensing in pulmonary artery myocytes. In addition, it is the most effective blocker of Kv4.2 channels described to date (IC_{50} 1.2 nM). HmTx1 shares sequence similarities with previously described potassium channel blocker toxins and HmTx2 with the calcium channel blocker toxin ω-GsTx SIA. Both HmTx1 and HmTx2 block potassium current associated with Kv2 subtypes in the 100-300 nM concentration range. HmTx2 appears to be a specific blocker of Kv2 channels while HmTx1 also blocks Kv4 channels, including Kv4.1 with the same potency. HmTx1 is the first described peptide effector of the Kv4.1 subtype. Those novel toxins are new tools for the investigation of the physiological role of the different potassium channel subunits in cellular physiology, similarly to the phrixotoxins which were recently used to elucidate the exact role of I_A-type I_{to} current in cardiac function.

Other tarantula toxins have also been found to act upon proton-gated cationic channels of the recently discovered ASIC family. Acid sensing is associated with nociception, taste transduction and perception of extracellar pH fluctuations in the brain. PcTx1, isolated from *Psalmopoeus cambridgei* venom potently blocks (IC_{50} = 0.9 nM) the ASIC1a subtype with high selectivity. As ASIC channels are highly expressed in both central nervous system neurons and sensory neurons from dorsal root ganglia, ASIC1a has become a powerful tool for the study of native ASIC1 currents underlied by the homomultimeric assembly of ASIC1a. PcTx1 will be an important tool for future studies of the involvement of ASIC channels in nociception, synaptic transmission and memory processes. Structure-activity studies on natural mutants will bring forward important clues about toxin-channel interaction and the mapping of the toxin receptor site.

Symposium 16 - Paper 2

MARTENTOXIN, A DISTINCT K^+ CHANNEL-BLOCKING LIGAND: PURIFICATION, GENOMIC ORGANIZATION, ELECTROPHYSIOLOGICAL AND BIOSENSOR BINDING CHARACTERISTICS

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Martentoxin, a novel K^+ channel-specific ligand purified from the venom of East-Asian scorpion has been characterized. The whole cDNA precursor sequence suggested that martentoxin was composed of 37 residues with a unique sequence compared with other scorpion neurotoxins. The genomic DNA of martentoxin showed an additional intron situated unexpectedly in the 5' UTR region, besides a common one located close to the C-terminal of signal peptide. The patch-clamp recording found that martentoxin could strongly block BKCa currents in RACCs. However, BKCa currents blocked by martentoxin can be fully recovered within 30s after wash, which is at least 10 times faster than that by charybdotoxin. In contrast, only 10% voltage-gated K^+ currents were depressed even at the dose of 300 nM martentoxin on either NG 108-15 cells or the DRG neurons. Biosensor binding assay showed a fast association rate and a slow dissociation rate of martentoxin binding on rat brain synaptosomes. The binding of martentoxin on rat brain synaptosomes could be inhibited regularly by charybdotoxin, and gradually by toosendanin in a concentration-dependent manner, but not by either apamin or BmP03. The results thus indicates that martentoxin may form a new group in the family of K^+ channel-blocking ligands.

Symposium 16 - Paper 3

κ-HEFUTOXIN1: A NOVEL TOXIN FROM THE SCORPION *HETEROMETRUS FULVIPES* WITH UNIQUE STRUCTURE AND FUNCTION

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An important and exciting challenge in the post-genomic era is to understand the functions of newly discovered proteins based on their structures. The main thrust is to find the common structural motifs that contribute to specific functions. Using this premise, here we report the purification, solution NMR and functional characterization of a novel class of weak potassium channel toxins from the venom of the scorpion *Heterometrus fulvipes*. These toxins, κ-hefutoxin1 and κ-hefutoxin2, exhibit no homology to any known toxins. NMR studies indicate that κ-hefutoxin1adopts a unique three-dimensional fold of two parallel helices linked by two disulfide bridges without any β–sheets. Based on the presence of the functional diad (Y^5/K^{19}) at a distance (6.0 ± 1.0 Å) comparable to other potassium channel toxins, we hypothesized its function as a potassium channel toxin. κ-Hefutoxin1 not only blocks the voltage-gated K^+-channels: *Kv1.3 and Kv1.2,* but also slows the activation kinetics of *Kv1.3* currents - a novel feature of κ-hefutoxin1 unlike other scorpion toxins, which are considered solely pore-blockers. Alanine mutants of the toxin failed to block the channels indicating the importance of the functional diad.

PARTIAL PROTEIN AND DNA SEQUENCES OF *LATRODECTUS HASSELTI, L. HESPERUS* AND *L MACTANS* LATROTOXINS: ARE THEY HOMOLOGOUS?

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Latrodectus spp. venoms exhibit similar clinical envenomation syndromes in humans and similar *in-vitro* and *in-vivo* activity. Venom toxicity is produced by vertebrate-selective α-latrotoxin (α-LTx) in humans and insect-selective α- and δ-latroinsectotoxins (LIT) in insects. These high molecular weight toxins (120-130 kDa) have been fully characterized from the venom of *L. tredecimguttatus* but, to date, no information exists on the structure of latrotoxins from other *Latrodectus spp.* Species-specific *Latrodectus* antivenoms have been shown to reverse clinical envenomation as well as *in-vitro* and *in-vivo* venom toxicity from geographically remote *Latrodectus spp.* This suggests that α-LTx exhibits some degree of homology across species (1).

Partial amino acid sequences were determined for α-LTx, α- and δ-LIT-like proteins from the venoms of *L. hasselti, L. hesperus* and *L. mactans*. Proteins were purified using sequential size-exclusion and anion exchange FPLC, followed by C_{18} RP-HPLC. N-terminal protein sequencing by Edman degradation was unsuccessful, most likely due to N-terminal blocking. Accordingly, toxins were subjected to tryptic digestion before MS/MS peptide sequencing was performed. Amino acid sequences were compared with those of respective latrotoxins from *L. tredecimguttatus* and assignment of isoleucine or leucine residues was made from the known sequences.

	MS/MS amino acid homology with *L. tredecimguttatus* latrotoxins		
Venoms	α-LTx	α-LIT	δ-LIT
L. hasselti	54/54 (100%)	150/151 (99%)	62/75 (83%)
L. hesperus	22/22 (100%)	-	135/148 (91%)
L. mactans	184/190 (97%)	-	-

Partial DNA gene sequences for *L. hasselti* α-LTx were derived from genomic DNA by polymerase chain reaction employing non-degenerate DNA primers designed from the known DNA sequence of *L. tredecimguttatus* α-LTx (2). This sequence [~540 bp] also revealed a high degree of homology (94%) with the published DNA sequence of α-LTx (2). These observations suggest that all latrotoxins from a range of *Latrodectus spp.* exhibit protein and gene sequence homology. This information may be helpful in elucidating comparative structure-activity relationships of these toxins.

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INSECTICIDAL COOPERATIVITY BETWEEN AMPHIPATHIC PEPTIDES AND NEUROTOXINS IN SPIDER VENOMS

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Five amphipathic peptides with antimicrobial, hemolytic and insecticidal activity were isolated from the crude venom of the wolf spider *Oxyopes kitabensis*. The peptides, named oxyopinins, are the largest linear cationic amphipathic peptides from the venom of a spider that have been chemically characterized at present. According to their primary structure oxyopinin 1 is composed of 48 amino acid residues showing extended sequence similarity to the ant insecticidal peptide ponericinl2 and to the frog antimicrobial peptide dermaseptin. Oxyopinins 2a, 2b, 2c and 2d have highly similar sequences. At least 27 out of 37 amino acid residues are conserved. They also show a segment of sequence similar to ponericinl2. Circular dichroism analyses showed that the secondary structure of the five peptides is essentially α-helical. Oxyopinins showed disrupting activities towards both biological membranes and artificial vesicles, particularly to those rich in phosphatidylcholine. Electrophysiological recordings performed on insect cells (sf9) showed that the oxyopinins produce a drastic reduction of cell membrane resistance by opening non-selective ion channels. Application of mixtures containing oxyopinins and spider neurotoxins to insect larvae showed a potentiation phenomenon, by which an increase lethality effect is observed. These results suggest that the linear amphipathic peptides in spider venoms and neuropeptides cooperate to capture insects efficiently.

STRUCTURE-FUNCTION STUDIES OF NEUROTOXIC PEPTIDES WITH OR WITHOUT ICK MOTIF PURIFIED FROM THE VENOM OF THE CHINESE BIRD SPIDERS

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Four neurotoxic peptides, containing 33-37 amino acid residues with three disulfide bonds, were purified from the venom of Chinese bird spider *S. huwena* and *S. hainana.* Huwentoxin-IV (HWTX-IV), hainantoxin-VI (HNTX-VI) and hainantoxin-III (HNTX-III) are highly potent neurotoxins which specifically inhibit the neuronal tetrodotoxin-sensitive (TTX-S) voltage-gated sodium channel (VGSC) in adult rat dorsal root ganglion (DRG) neurons with the IC_{50} value of 30 nM, 31 nM and 1.14 nM respectively. HWTX-VI, HNTX-VI and HNTX-III were proved to have no significant effect on tetrodotoxin-resistant (TTX-R) VGSC. These three toxins seem to be the site-1 toxins affecting the sodium channel through a mechanism quite similar to that of TTX. They suppresse the peak sodium current without altering the activation or inactivation kinetics. The three-dimensional solution structure of the three toxins have also been elucidated via standard homonuclear nuclear magnetic resonance (NMR) methods, yielding three new member of the inhibitor cystine knot (ICK) motif family.

Huwentoxin-II (HWTX-II) is an insecticidal peptide purified from the venom of spider *Selenocosmia huwena* with a unique disulfide bond linkage as I-III, II-V and IV-VI. The three dimensional structure has been determined using 2D ^{1}H-NMR. The structure of HWTX-II contains two turns (C4-S7 and K24-W27) and a double-stranded antiparallel β-sheet (W27-C29 and C34-K36). Comparison of the structure of HWTX-II and the inhibitor cystine knot (ICK) motif, which is widely adopted by spider and cone shell toxins and inhibitory polypeptides, shows that HWTX-II adopts a scaffold different from the ICK motif, because HWTX-II does not form a cystine knot due to the unique disulfide bridges linkage. Moreover, it is found that the double-stranded β-sheet directed by two disulfide bonds (II-V and IV-VI) in HWTX-II is conserved in the ICK motif molecules, the α/β folded scorpion toxins, the snake toxins and the growth factor cystine knots molecules. The conservation in these molecules adopting different overall motifs suggests that they may have evolved from the same simple structural ancestor characterized by a β-hairpin directed by two disulfide bridges.

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Symposium 17 - Paper 2

MOLECULAR CHARACTERIZATION OF THE INSECTICIDAL NEUROTOXIN J-ACTX-Hv1c

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In addition to destroying an estimated 20–30% of the world's food supply, arthropod pests are responsible for the transmission of many new and reemerging human diseases . These arthropods have generally been controlled by spraying broad-spectrum chemical insecticides. However, the emergence of insecticide-resistant insect populations, as well as increasing disquiet about the environmental and human health risks associated with certain agrochemicals, has stimulated the search for new arthropod-control strategies.

The Janus-faced atracotoxins (J-ACTXs) are a family of insect-specific excitatory neurotoxins that we isolated from the venom of Australian funnel-web spiders [2]. In addition to a strikingly asymmetric distribution of charged residues, from which their name is derived, these toxins contain an extremely rare vicinal disulfide bond [2]. In order to shed light on the mechanism of action of these toxins, and to enhance their utility as lead compounds for insecticide development, we developed an efficient *Escherichia coli* expression system for production of recombinant J-ACTX-Hv1c so that structure-function relationships could be elucidated using site-directed mutagenesis. We will present data on the structure and function of a complete panel of alanine-scan mutants, as well as a series of additional point mutants designed to probe the role of individual chemical moeities in functionally important residues [3]. We will also present data showing that the N-terminus is proximal to the binding site whereas the C-terminal residues are dispensible [4]. These studies have allowed us to compile the first high-resolution map of a spider toxin pharmacophore.

While the pharmacophore revealed by site-directed mutagenesis has provided clues as to the likely target of the J-ACTXs [3], their molecular target remains unidentified. We are employing an unbiased genetic screen using transgenic *Drosophila* that express a gene for J-ACTX-Hv1c under the control of the Hsp70 heat-shock promoter. Mating transgenic females with EMS-mutagenized males, followed by heat shock of the F1 progeny, should result in survivors having a resistance mutation that can be mapped to reveal putative toxin target gene(s). In addition to genetic methods we are employing various biochemical means to identify the target of these unique toxins.

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Symposium 18 - Paper 1

BARNES AND SOUTHCOTT — TWO TRUE PIONEERS

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Medical toxinology in the Asia-Pacific region has owed much to the work of enthusiastic individuals pursuing a fascination with toxinology as a vocation secondary to that of their primary profession.

Two such pioneers were Dr Jack Barnes (1922 – 1985) and Dr Ronald Vernon Southcott (1918 – 1998). Both graduated as medical practitioners and both worked essentially alone and unsupported in the dedicated pursuit of knowledge in the area of marine toxinology. Both have been immortalised by the naming of creatures in their honour — Carukia barnesi, the Irukandji jellyfish and Microtrombidium southcotti, a small but highly ornamented mite which characterised much of the passion of one of the nation's leading acarologists.

This Congress salutes their memory and pays tribute to their pioneering contributions.

Dr Jack Barnes was a colourful Cairns general practitioner who tackled the enigmatic problems of jellyfish stings and deaths in north Queensland waters. He first suggested to troubled lifesavers in north Queensland that two pairs of pantyhose, worn as a safety suit from the waist down, would enable them to enter dangerous waters where lethal jellyfish were thought to swim; and to drag nets such that the hitherto unnamed and unidentified jellyfish might be caught and subjected to formal study. He caught many specimens of what came to be called Chironex fleckeri, this latter named in 1956 by Dr Ron Southcott, working in Adelaide. It was Barnes who tackled the enigma of the cramping and collapse syndrome which afflicted swimmers in north Queensland waters; and he first caught and tested a small carybdeid jellyfish to see if it was associated with the Irukandji stinging. He experimented on himself and confirmed the aetiology of the syndrome.

Jack Barnes' fieldwork and Ron Southcott's meticulous laboratory taxanomic and anatomical studies revealed much about the world's most venomous creature, Chironex fleckeri, literally the "assassin in the sea". Recognising the need for pure Chironex venom, Jack Barnes "milked the tentacles, and ascertained a way of extracting as pure a venom as possible from its source". After much trial and error, he collected Chironex venom using an amniotic membrane. One of his obituaries described Jack Barnes as "irritable and belligerent as many short men can be, demanding and critical, unsociable and rude, scathing of politicians and unco-operative with authority...but under that gruff

exterior was a kindly and compassionate nature.. his deeds gave him away and his many faithful patients and his fellow medicos all knew that he practised medicine with skill and honesty and a proper compassion for the sick and frightened.. and it was due to his efforts that the science of marine toxinology was significantly advanced".

Dr Ronald Southcott was born in Adelaide and apart from a period spent in the Northern Territory in uniform (a very unsoldierly Captain Ron Southcott), he spent almost all of his life based in Adelaide. He undertook major epidemiological work into the epidemic patterns of poliomyelitis and was an indefatigable Deputy Director of the Department of Veterans Affairs in South Australia. His fame, however, is that as a naturalist and acarologist. He wrote more than 70 papers exclusively in acarology and was awarded the Degree of Doctor of Science by the University of Adelaide in 1962 for his "Studies on the Systematics and Biology of the Acarina". He was responsible for an epic programme of research on ectoparasitic mites and one of his obituaries notes that he "will be remembered for his many attempts, some successful, to rear Trombidiid mites through their lifecycles... and thus to break through the frustrating scientific barrier between the identification of the larvae on the one hand and adults on the other". Dr Ronald Southcott brought an extraordinary depth of erudition to many fields of natural history, particularly that relating to tropical medicine and marine toxinology. His detailed microscopy and taxonomy of Chironex and his work on toxic and venomous marine and freshwater fish, also added considerably to knowledge. One of his unfinished and unresolved passions, often recounted to the authors, was to "get to grips with the real problems of jellyfish taxonomy in Australia". In all, Ron Southcott published 234 major papers and works in refereed published journals.

Nearly all the research of these two trailblazers was self-funded. They established their own laboratories in their own homes and their research became enmeshed as an integral part of their domestic, personal and professional lives. Thus is the spirit of the true pioneer.

CONOTOXINS AND CONE SNAILS: EVOLVING A SUCCESSFUL NEUROPHARMACOLOGICAL STRATEGY

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The venomous cone snails typically contain ca. 100 small, structured peptides in their venoms (conotoxins), which they use primarily to capture prey, but also to defend against predators and deter competitors (1-3). Remarkably, almost no overlap occurs between peptides of different *Conus* species; thus, the 500 *Conus* species produce about 50,000 different peptides.

Cone snails may be viewed as highly specialized for using neuropharmacology for their interactions with other animals (2). Their overall strategy has parallels with the drug development strategies of large pharmaceutical firms that use combinatorial libraries to generate a lead compound for drug development and sophisticated medicinal chemistry to further refine the lead. For the cone snails, the equivalent of a combinatorial library is hypermutation of venom peptides during speciation (2,4), and the equivalent of medicinal chemistry is an extensive series of post-translational modifications (5). Our present understanding of these aspects of *Conus* peptide biology will be reviewed. The corollary to employing multiple pharmacological agents simultaneously for a specific physiological end-point (6) is that each individual venom peptide must be very specifically targeted; the mechanistic basis for the exquisite molecular recognition that has evolved in cone snails will be discussed (7).

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Symposium 18 - Paper 3

ANALOGUES OF ENDOGENOUS NEUROPEPTIDES IN *CONUS* VENOMS

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Not all peptides found in *Conus* venoms have structures unique to *Conus* species. Several conopeptides with strong sequence similarity to endogenous neuropeptides have been isolated. The first discovered were Lys-conopressin-G from *Conus geographus* and Arg-conopressin-S from *C. striatus* (1). The conopressins are obviously members of the vasopressin-oxytocin peptide family and proved to be agonists of the vasopressin receptor. Other examples include contulakin-G, an O-glycosylated neurotensin analogue isolated from *C. geographus* (2) and conorfamide, a new *Conus* peptide isolated from *C. spurius*, which was shown to be a member of the RFamide neuropeptide family (3).

A novel 84-amino acid *Conus* peptide isolated from *C. radiatus* venom has been found to contain 14 Cys residues in a distinctive disulfide framework and sequence reminiscent of the neurophysin peptide family (4). Conophysin-R is the first neurophysin-like peptide that has been found in any venom and it is the first neurophysin family member isolated and biochemically characterized from an invertebrate source. The new polypeptide has some unique features among neurophysin-like peptides; it is the only member of the group with less than 7 amino acids between the first two Cys residues and it has an exceptionally long loop between Cys 9 and Cys 10. One possible reason for its presence in *C. radiatus* venom is that it is derived from an endogenous neurophysin. It is also conceivable that the neurophysin disulfide framework may serve as a conserved scaffold for peptides that have diverged to the point where their functional role in the venom is unrelated to the endogenous functions of neurophysins. As new species are examined, it is likely that many more endogenous peptide analogues will be discovered among the ~50,000 peptides in *Conus* venoms

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Symposium 18 - Paper 4

"WHAT ON EARTH IS CIGUATERA?"

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Ciguatera is a pleomorphic syndrome consisting of a range of gastrointestinal, neurological and cardiovascular signs and symptoms that follow the consumption of warm-water marine fish contaminated with sodium channel activator toxins known as ciguatoxins. The disease is rarely fatal and its severity and duration may be reduced with intravenous mannitol. At least two structurally distinct families of ciguatoxins, one from the Pacific and one from the Caribbean, have been identified. A third family of ciguatoxins has recently been identified in ciguateric reef fishes of the Indian Ocean. All these ciguatoxins (CTX) most likely arise from certain strains of the benthic dinoflagellate, *Gambierdiscus toxicus*. Following blooms of *G. toxicus*, these toxins accumulate in fish through marine food chains to levels that affect human health. Factor(s) influencing such bloom formation are unclear. P-CTX-1, the most potent sodium channel toxin known, is the major toxin contributing to ciguatera caused by carnivorous fish in the Pacific, causing human poisoning at levels of 0.1 ppb (10^{-10} mole P-CTX-1/kg) and above. Toxins produced by other benthic dinoflagellates, including okadaic acid and maitotoxin, have no proven role in ciguatera. The mouse assay is presently used to assess levels of ciguatoxin in fish extracts. Other *in vivo* assays, including the chicken, mongoose, mosquito, brine shrimp and diptera larvae assays, are less widely used. *In vitro* cell-based assay, which measure the effects of ciguatoxin-induced sodium channel opening or the inhibition of [^{3}H]-brevetoxin binding, are more sensitive than *in vivo* methods and have the potential to replace the mouse assay. Analytical detection methods, in particular HPLC/tandem ionspray mass spectrometry, are also under development. Antibody-based assays offer hope for as cost-effective screens for ciguateric fish, but presently appear to lack the necessary specificity and sensitivity. A major advance in the management of ciguatera will come when a validated screen is commercially available. This presentation will discuss advances in our understanding of ciguatera since "Hank" Banner initiated modern research into the problem in Hawaii in the late 1950s, and hopefully answer more on the question "what on earth is ciguatera?" posed by the late Dr Struan Sutherland in 1986.

Symposium 18 - Paper 5

DISCOVERY OF ANALGESIC CONOTOXINS

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The Conidae are a group of venomous marine gastropods (cone snails) that are found in tropical and sub-tropical waters. Venoms contained in the venom ducts of *Conus* species are delivered by a harpoon to rapidly immobilise prey. Mass profiling of these venoms has yielded an array of low molecular weight microproteins, the conotoxins, which typically contain polypeptide chains of 10-40 amino acids which are highly constrained by two to four disulfide bridges.

Of particular interest is the high stability, potency and exquisite selectivity of different conotoxins for ion channels, transporters and G-coupled protein receptors. In this presentation I will describe the discovery and characterisation of two classes of conotoxins that have potent analgesic properties.

Symposium 19 - Paper 1

CONANTOKIN-L, A NEW NMDA RECEPTOR ANTAGONIST FROM *CONUS LYNCEUS*

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The conantokins comprise a family of conotoxins which induce sleep in mice younger than two weeks and hyperactivity in older mice. These peptides are found to selectively bind to N-methyl-D-aspartate receptor. A new conantokin, conantokin-L from *C. lynceus*, has been identified from a cDNA clone, and the translation product was synthesized. It has extensive homology with conantokin-R from *C. radiatus*, except for the C-terminal amino acids. Conantokin-L appears almost as potent as conantokin-R in *in vivo* and NMDA receptor binding assays. However, it is less potent as an anticonvulsant, with a protective index of 1.2 in the audiogenic mouse model. The results indicate that the C-terminal sequences of conantokin-L and conantokin-R may determine their anticonvulsant potency.

Symposium 19 - Paper 2

α-CONOTOXINS FROM *Conus anemone*

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α-conotoxins present in the venom of marine snails of the genus *Conus* are known to interact selectively with different forms of the nicotinic acetylcholine receptor (nAChR). The diversity of sequences across all the α-conotoxins is striking given the conservation of their cysteine framework. The selectivity of α-conotoxins for 'muscle-type' or 'neuronal-type' nAChRs is determined principally by their loop size (3:5 "muscle-type" or 4:7 "neuronal-type") and by the hydrophobicity of certain amino acids included in the second loop (Broxton *et al* 2000).

In this study, α-conotoxins were identified in two ways. We have used PCR based techniques to identify a suite of α-conotoxins expressed in the venom duct of *C. anemone,* a vermivorous *Conus* from the Gulf of St. Vincent in South Australia. We then synthesized the deduced α-conotoxins and characterized their biological activity. Equivalent α-conotoxins were also isolated from *C. anemone* duct venom by RP-HPLC. The masses of these peptides were determined by MALDI-TOF MS and compared to the calculated mass of sequences determined by PCR. Several of the conotoxins isolated from the venom were post-translationally modified *in vivo.* This was confirmed by tandem MS.

The biological activity of the peptides isolated from the venom was compared with the activity of the peptides synthesized without post-translational modifications. The peptides were assayed for activity on *neuronal-type* nicotinic acetylcholine receptors in monolayer cultures of bovine adrenal chromaffin cells. The principal α-conotoxin, An1.1 (1-10 μM), competitively inhibited the release of catecholamines evoked by nicotine (1 μM) in a concentration-dependent manner, but had no effect on the release evoked by 56mM K^+, indicating an action at the ligand-gated ion channel rather than on voltage-gated ion channels. In addition, α-conotoxin An1.1 (10μM) protected against nicotinic receptor desensitization caused by high concentrations (>20μM) of nicotine. This work provides the first structural identification and functional characterization of α-conotoxins from *Conus anemone,* a vermivorous cone from temperate waters of southern Australia.

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**D.S. is an APG award scholar. We thank the South Australian Shell Club for field advice.*

AFFINITY CHROMATOGRAPHY ON CM-PAPAIN-SEPHAROSE AS A METHOD FOR PURIFICATION OF RECOMBINANT SAXIPHILIN AND RELATED SAXITOXIN-BINDING PROTEINS

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Saxiphilin is a 91 kDa plasma protein from the North American bullfrog (*Rana catesbeiana*) that specifically binds saxitoxin (STX) and various STX analogs with low nanomolar affinity (1). STX and certain STX analogs are causative agents of paralytic shellfish poisoning (PSP) due to consumption of mussels and clams contaminated with this lethal microalgal toxin. Saxiphilin and related proteins may thus serve as useful protein-based reagents for detection of STX in seafood and water samples as an alternative to live mouse bioassay. Saxiphilin is a transferrin-like molecule with a unique insertion of two tandem thyroglobulin type-1 protein modules (Thyr-1), a family of ~60-80 residue sequence motifs. Many Thyr-1 modules are now recognized as specific inhibitors of the papain family of cysteine proteinases (2). We have taken advantage of the specific interaction of saxiphilin and papain (K_i = 1.7 nM) to develop affinity-based methods for improved isolation of recombinant saxiphilin and similar STX-binding activity in animal plasma. Carboxymethyl-papain-Sepharose was prepared by inactivating papain with excess iodoacetic acid and coupling to CNBr-Sepharose. Physical association was monitored by titration of saxiphilin with increasing aliquots of Cm-papain-Sepharose. After removal of Sepharose by centrifugation, SDS-PAGE revealed quantitative depletion of saxiphilin from the supertantant. Affinity chromatography of crude insect cell media containing recombinant saxiphilin on Cm-papain-Sepharose provided effective purification of saxiphilin after washing and elution at pH 11. [^{3}H]STX-binding activity in plasma of the marine toad, *Bufo marinus*, was also rapidly adsorbed on Cm-papain-Sepharose, eluted at pH 11, and recovered in active form after adjustment to neutral pH.

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SAXITOXIN BINDING PROTEINS IN THE BLOOD: A MEANS OF DETOXIFICATION OR DECOY RECEPTORS?

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Until several year's ago, the voltage gated sodium channel (Na channel) was the only known receptor for the microalgal toxin, saxitoxin (STX). There are isoforms of this ion channel with much reduced sensitivity to STX, such as that from mammalian heart, which can be attributed to simple amino acid sequence changes. At physiological pH, STX possesses two positively charged guanidino groups both of which are involved in Na channel binding of STX, probably by direct charge-charge interaction with acidic amino acids. Two hydrophilic receptors for STX have now been discovered from the circulatory fluids of various higher animals, all of whom produce STX sensitive Na channels. The first, dubbed saxiphilin, is a relative of the iron-binding transferrin proteins isolated from the North American bullfrog. It binds only STX and its chemical relatives and not tetrodotoxin (TTX), the pufferfish poison which directly competes with STX for the same binding site on the Na channel. Proteins with similar STX selectivity and hydrophilicity to bullfrog saxiphilin have been found in amphibia, fish, reptiles and arthropods. Only one of the STX guanidino's is important for binding to saxiphilin and acidic amino acids are again necessary. A hydrophilic TTX binding protein from the blood of pufferfish has now been discovered that also binds STX. These three classes of STX binding proteins, delineated by their STX/TTX binding abilities and hydrophilic/hydrophobic nature, bind STX in the femptomolar to nanomolar affinity range and exhibit no amino acid sequence homology. Their production in many higher vertebrates and invertebrates is a curious biological mystery considering that STX production has only been confirmed in dinoflagellates and blue-green algae. Do these hydrophilic STX-binding proteins prevent STX from exerting its action upon the Na channel? Apart from the puffer-fish and some toxic xanthid crabs, none of the other animals that possess these hydrophilic toxin binding proteins are known to harbour or bioaccumulate STX. Do these proteins then bind a yet to be discovered chemical related to STX? And could this hypothetical compound play a more complex physiological role that can be likened to a hormone or cytokine? If so, then this co-occurrence of a circulatory binding protein which binds a ligand that can also bind to a membrane bound receptor has parallels with the human interleukin system. Excess interleukin production in certain human conditions is dealt with by cells expressing an interleukin receptor isoform lacking the membrane anchor. These so-called decoy receptors then bind the excess interleukin in the extracellular fluids and prevent it from exerting a biological effect.

TWO-STEP MEMBRANE BINDING OF EQUINATOXIN II, A PORE-FORMING TOXIN FROM THE SEA ANEMONE *Actinia equina*, INVOLVES AN EXPOSED AROMATIC CLUSTER AND A FLEXIBLE HELIX

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Equinatoxin II (EqtII) is a potent cytolysin isolated from the sea anemone *Actinia equina*. It belongs to a homologous group of proteins called actinoporins. Actinoporins are rich in β-sheet, they have high pI, Mr=20 kDa and their activity can be prevented by preincubation with sphingomyelin. They do not show any similarity to other pore-forming proteins and might as well represent a novel cytolytic toxin group. The recently solved 3D structure provided a structural framework for functional studies, which aim to understand the steps of pore formation (1,2). Current hypothesis proposes that the initial binding of these toxins is promoted by an aromatic rich cluster on the surface of the molecule (3). In the next step, the N-terminal helix translocates from the body of the molecule to the membrane and, finally, traverses the lipid bilayer forming the cation selective pore.

Interaction of EqtII and its tryptophan mutants with the membranes was studied by surface plasmon resonance (SPR) on a Biacore machine. The binding is a two-step process, separately mediated by two regions of the molecule. An exposed aromatic cluster involving tryptophans 112 and 116 mediates initial attachment, which is prerequisite for the next step. Sterical shielding of the aromatic cluster from solvent or mutation of Trp112 and 116 to phenylalanine significantly reduces the toxin-lipid interaction. The second step is promoted by the N-terminal amphiphilic helix, which translocates into the lipid phase. Both steps were distinguished by the use of a double cysteine mutant having the N-terminal helix fixed to the protein core by a disulfide bond. The kinetic of membrane binding derived from the SPR experiments could be fitted to a two-stage binding model: initial binding to the membranes and insertion of the N-terminal helix into the membrane which provides additional contacts with the membrane and leads to a stable insertion.

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ASPECTS OF THE WORK OF DR ROBERT ENDEAN (1925-1997): AUSTRALIAN MARINE BIOLOGIST AND TOXINOLOGIST.

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For more than thirty years Bob Endean studied marine animals potentially dangerous to man, which were collected, in the main, from Queensland waters. For each group under examination, he sought to identify the potentially lethal species, to understand the structure of the venom gland, the mode of delivery of the venom, the pharmacological effect of the venom on excitable tissues, and if possible, the biochemistry of the toxic material and its mode of action.

In 1962 Endean and coworkers began an investigation of crude venom from a number of piscivorus species of *Conus* snails. This work demonstrated for the first time the presence of multiple toxins in the venoms of different species, and also pointed to a very high specificity of toxic action. In his study of nematocysts from the box-jellyfish *Chironex fleckeri*, Endean devised a procedure to obtain nematocyst extract free from tentacle material. This led to the isolation of two potent high mol. wt myotoxins considered to be the principal toxins of nematocyst envenoming. He initiated studies of ciguatoxin in Queensland coastal fish, and of paralytic shellfish toxins in reef crabs from Australian waters. Endean's knowledge of the inter-relationships of toxic marine communities, particularly in the Great Barrier Reef, enabled him to highlight ecological dangers and eventually to galvanize action to protect these communities.

Symposium 20 - Paper 2

A STRUCTURALLY CONSERVED K^+-CHANNEL BLOCKING PEPTIDE IN DIFFERENT *CONUS* VENOMS

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From the venom of the cone snail *Conus virgo* (Philippines) a peptide was isolated, named ViTx, which blocked K^+-channels of the Kv 1.1 and 1.3 type, but not of Kv 1.2 type. The peptide is composed of a chain of 35 amino acids cross-linked by four disulfide-bridges. Based on the signalling sequence of this peptide degenerated primers were constructed in an attempt to identify similar peptides in other *Conus* venoms. By PCR-screening of cDNA from the venom glands of five *Conus* species a nucleotide sequence was detected in *Conus vexillum* exhibiting almost complete homology to that of ViTxt. Elecrophysiological experiments (voltage clamp) on K^+-channels expressed in *Xenopus* oocytes comfirmed the presence of a component with K^+-channel blocking activity in this venom. In other species such as in *Conus miles, C. imperialis, C. capitaneus* and *C. striatus* the same peptide seems also to be a minor venom constituent. Despite the considerable hypervariability of *Conus* toxins, members of this particular peptide family may occur with almost identical structural entities and biological activities in various *Conus* venoms.

Symposium 20 - Paper 3

ECOLOGICAL REASONS FOR VARITATIONS IN VENOM LETHALITY IN TWO SPECIES OF AUSTRALIAN BOX JELLYFISH, *CHIRONEX FLECKERI* AND *CHIROPSALMUS* SP.

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In the coastal waters off northern Australia there are two major species of large box jellyfish, *Chironex fleckeri* and *Chiropsalmus* sp. *Chiropsalmus* sp. has never been recorded as causing a fatality, while numerous deaths have been attributed to *Chironex fleckeri.* In *Chiropsalmus* sp., a species that feeds exclusively on shrimp, no changes in the ratio of the three different groups of nematocyst present were detected with the change in size of the individual animals. In *Chironex fleckeri*, a box jellyfish responsible for over 60 deaths in Australia alone, the ratio of different types of nematocysts in the cnidome for small animals (less than 40mm) was similar to that of *Chiropsalmus* sp. However, with an increase in body size in *C. fleckeri*, the nematocyst ratio changed, with mastigophores (containing the lethal venom component for prey) increasing in proportion dramatically. The change in nematocyst ratio is positively correlated with size and a change in prey. Small *C. fleckeri* fed exclusively on prawns, medium sized animals fed on fish and prawns and large animals fed predominantly on fish. An increase in the proportion of mastigophores in *C. fleckeri* may also be responsible for why this species has caused numerous human fatalities, while the Australian *Chiropsalmus* sp. has not.

Symposium 20 - Paper 4

PUFFER FISH POISONING : A POORLY RECOGNISED AND POTENTIALLY LIFE-THREATENING CONDITION IN AUSTRALIA

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Puffer fish poisoning results from ingestion of tetrodotoxin (TTX), present in high concentrations in the liver, intestines and skin of the fish. Although TTX poisoning is reasonably common in some parts of the world, it only occurs sporadically in Australia, with only 15 cases reported.[1] The *in vitro* effects of TTX are well characterised, but less is known about the *in vivo* effects in humans. This study describes for the first time clinical, neurophysiological and nerve excitability changes in a group of patients following accidental ingestion of puffer fish.

7 adults and 2 children consumed a soup made from approximately 30 puffer fish. The seven adults presented to hospital with nausea and vomiting, perioral and lingual numbness, dysaesthesia of the extremities, dizziness and gait ataxia. The onset of these symptoms was about 90 minutes after ingestion. They had no abdominal pain or diarrhoea. One patient reported respiratory distress. Neurological examination revealed weakness, more marked in the upper than the lower limbs, and marked ataxia. The effects gradually resolved over 48 hours but slight weakness and ataxia of the lower limbs remained. These resolved completely over the next week. Neurophysiological investigation, undertaken in 4 patients, demonstrated marked abnormalities in parameters dependent on Na^+ channel function when compared to normal subjects[2]. The stimulus current required to generate a test potential was increased [12.2 ± 3.4mA in patients, 4.6 ± 0.2mA in controls (mean ± SEM); $p<0.0001$]; latency was prolonged [8.0 ± 0.2ms in patients, 6.6 ± 0.1ms in controls; $p<0.0005$]; and relative refractory period increased [3.5 ± 0.05ms in patients, 3.1 ± 0.05ms in controls; $p<0.05$]. These findings are consistent with blockade of Na^+ channels by TTX. While the majority of patients required hospital admission for 48 hours, there were no long-term complications. It is important that TTX poisoning is recognized early in regions like Australia, so patients can be observed in an appropriate critical care setting.

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IDENTIFICATION OF TOXIN AND FISH SPECIES IN FRAUD DRIED MULLET ROE IMPLICATED IN FOOD POISONING

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There was a victim of neurotoxic food poisoning from a fraud dried mullet roe in Kaohsiung, Taiwan, in March 2001. The victim exhibited neurotoxic symptoms including general discomfort, diaphoresis, dyspnea, respiratory failure and decreasingly blood pressure. The small residue of fraud dried mullet roe retained by the victim was assayed for toxicity and mitochondrial DNA. The toxicity of the fraud dried mullet roe was 3450 mouse units per gram (MU/g). The toxin from fraud dried mullet roe was partially purified and identified as tetrodotoxin and derivative. The sequence of the 376-nucleotide region in the cytochrome *b* gene of the mitochondrial DNA exhibited the same genotype and the same restriction site for *Hinf*I as that of the toxic puffer fish *Lagocephalus lunaris*. Therefore, the species of the fraud dried mullet roe was identified as *Lagocephalus lunaris* and its causative agent was identified as tetrodotoxin in this food poisoning.

SOLUTION STRUCTURE OF μ-CONOTOXIN PIIIA, A PREFERENTIAL INHIBITOR OF PERSISTENT TTX-SENSITIVE SODIUM CHANNELS

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Voltage-sensitive sodium channels (VSSCs) underlie the influx of sodium ions responsible for action potentials in excitable cells. Based on their susceptibility to block by tetrodotoxin (TTX), VSSCs can be divided into TTX sensitive (TTX-S) and TTX-resistant (TTX-R) classes. Members of both classes share considerable sequence homology and are closely related structurally. A number of these VSSC subtypes are implicated in clinical states such as pain, stroke and epilepsy. Persistent (non-inactivating) forms of the TTX-S sodium channel current which underlie repetitive firing have less well-defined origins, but may involve $Na_v1.3$ or $Na_v1.6$ and are enhanced by hypoxia and nitric oxide. Most TTX-S sodium channels types have a heterogeneous distribution in human brain.

VSSCs are inhibited by local anaesthetics and modulated by toxins that act at one inhibitory site (Site 1) and at least four other sites that result in excitatory actions. μ-Conotoxins from the venom of marine cone snails act selectively to occlude the pore of the VSSC by competing with TTX and saxitoxin (STX) for binding to Site 1 in the P-loop region of the α subunit. To date, sequences for four members of the three-loop μ-conotoxin class have been published. GIIIA, GIIIB and GIIIC from *Conus geographus* venom are potent blockers of skeletal muscle, but not neuronal VSSCs. The three-dimensional (3D) structures of selected μ-conotoxins have been used to describe the architecture of the outer vestibule of the VSSC. The most recently described member of this class is μ-conotoxin PIIIA from *C. purpurescens*. PIIIA is notable for its ability to inhibit neuronal as well as muscle TTX-S sodium channels, and to discriminate among VSSCs in rat brain. Thus PIIIA is the first peptide toxin for investigating the architecture of Site 1 of neuronal VSSCs.

To further investigate the potential of PIIIA as a probe of VSSCs, we determined its structure by ^{1}H NMR spectroscopy and characterised its mode of action on native tissues using electrophysiological and ligand binding approaches. Synthetic forms of μ-conotoxins PIIIA and PIIIA(2-22) were found to inhibit tetrodotoxin (TTX)-sensitive VSSC current but had little effect on TTX-resistant VSSC current in sensory ganglion neurons. In rat brain neurons, these peptides preferentially inhibited the persistent over the transient VSSC current. Radioligand binding assays revealed that PIIIA, PIIIA(2-22) and μ-conotoxin GIIIB discriminated among TTX-sensitive VSSCs in rat brain, that these and GIIIC discriminated among the corresponding VSSCs in human brain, while GIIIA had low affinity for neuronal VSSCs. ^{1}H NMR studies found that PIIIA adopts two conformations in solution due to *cis/trans* isomerisation at hydroxyproline8. The major *trans* conformation results in a 3D structure that is significantly different from the previously identified conformation of μ-conotoxins GIIIA and GIIIB that selectively target TTX-sensitive muscle VSSCs. Comparison of the structures and activity of PIIIA to muscle-selective μ-conotoxins provides an insight into the structural requirements for inhibition of different TTX-sensitive sodium channels by μ-conotoxins.

Poster Abstracts

Poster 1-1

CLONING AND EXPRESSION OF A SYNTHETIC GENE FOR OHANIN: A NOVEL PROTEIN FROM KING COBRA VENOM

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We have recently purified and determined the complete amino acid sequence of a novel protein, Ohanin (SP: P83234), from *Ophiophagus hannah* venom (Bali King Cobra). Ohanin is small (11951.47±0.67Da) globular protein toxin containing 107 amino acids. Amino acid sequence of the protein shows 95% homology to Thai Cobrin, a similar isoform from *Naja naja kaouthia* (SP: P82885). Biological significance of the protein is not clearly understood to-date. Since the natural abundance of the protein is low (~2.5 mg/g venom), an *E. coli* expression system was designed to obtain large quantities of the protein. The native amino acid sequence was reverse translated to *E. coli* preferential codons and the resulting nucleotide combinations was manipulated in order to evenly distribute 10 unique restriction sites along the length of the gene. The synthetic gene was cloned into a T7 promoter-controlled plasmid (pET-32a) and expressed as a hybrid protein fused with His-tag at the N-terminal.

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Poster 1-2

NUCLEOTIDE SEQUENCES OF NEUROTOXIN GENES ISOLATED FROM *BUNGARUS CANDIDUS* SHARE HOMOLOGY WITH BUNGAROTOXIN GENES OF *BUNGARUS MULTICINCTUS* BUT NOT *BUNGARUS FASCIATUS*

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According to the similarity between Taiwanese many – banded krait (*Bungarus multicinctus*) and Malayan krait (*Bungarus candidus*), the highly conserved nucleotide sequences of α - and β - bungarotoxins from *B. multicinctus* were designed as primers to amplify gene encoding neurotoxins from *B. candidus* and *B. fasciatus*. The amplified products were sequenced and the results showed that the nucleotide sequences of neurotoxin genes from *B. candidus* share 95 – 100 % identities with α - and β - bungarotoxin genes. Furthermore, polyspecific antivenom of *B. multicinctus* and *Naja naja atra* could cross – react with many proteins from venom of *B. candidus*. They cross – reacted only with a few proteins of *B. fasciatus* venom using Western blot. Specific antivenom to *B. candidus* is not currently available. Our data suggest that animal experiments are indicated to evaluate *B. multicinctus* antivenom's capacity to neutralize intoxication from *B. candidus*.

Poster 1-3

ANALYSIS OF THE TRANSCRIPTOME FROM *Bothrops insularis* VENOM GLANDS: IDENTIFICATION OF NEW PROTEINS THROUGH THE GENERATION OF EXPRESSED SEQUENCE TAGS (ESTs)

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Expressed Sequence Tags are DNA sequences obtained from 5′ or 3′ ends of cDNA clones randomly chosen which are used in the characterization of gene expression (transcriptome) of target tissues. In this project, we aimed first to generate an EST database to catalogue the most abundant mRNAs expressed in the venom glands of the pitviper snake *Bothrops insularis – Viperidae*. In a second step, some of the most biologically relevant cDNAs identified were expressed in *E. coli* for biochemical and biological characterization of the recombinant proteins.

Sequences from 610 independent clones from a plasmidial cDNA library were assembled in 297 clusters and analyzed by similarity against GenBank, revealing the putative identification of 210 distinct gene products and 87 distinct unknown proteins. Toxin sequences corresponded to 56% of all transcripts (85 clusters), being the metalloproteinases (23%) and the bradykinin-potentiating peptides (BPPs) (11%) the major components. Other important toxins were identified, including serine proteinases, C-type lectins, L-amino acid oxidase and a new abundant transcript similar to human Vascular Endothelial Growth Factor. This factor was expressed in *E. coli*, and characterized, showing vascular permeability increase ability in mice (1). In addition, an anti-svVEGF was produced and showed the ubiquitous distribution of this new toxin in snake venoms, even in *Elapidae* species. Among the 125 clusters matching cellular proteins, the major part represents molecules involved in gene and protein expression, notably in disulfide bond assembly, reflecting a high specialization of this tissue for toxin synthesis. A cDNA, coding for a EF-hand protein similar to calmodulin, was also expressed in *E. coli* and its secondary structure and Ca^{2+} binding ability were demonstrated by circular dichroism (CD). This protein is specifically expressed in the snake venom glands but not expressed in the other snake tissues or in the rat salivary gland. It is also not detected in the venom, constituting a cellular component probably involved in the secretor processes. An unusual representation of retrotransposon-like sequences was also found and could be related to the occurrence and diversity of many paralogous forms of toxins in venom gland.

In conclusion, our *B. insularis* dbEST allowed the identification of the most common classes of toxins present in *Viperidae* venoms, which parallels the complex hemorrhagic effects evoked by the venom on the prey. In addition, it provides the first comprehensive set of reptilian gene sequences described so far.

This work is supported by FAPESP, CNPq and Fundação Butantan

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Poster 1-4

MOLECULAR CLONING AND EXPRESSION OF A FUNCTIONAL SNAKE VENOM VASCULAR ENDOTHELIAL GROWTH FACTOR (svVEGF) FROM THE *Bothrops insularis* PITVIPER. A NEW MEMBER OF THE VEGF FAMILY OF PROTEINS

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During the generation and analysis of abundant Expressed Sequence Tags (ESTs) from the Viperidae snake *Bothrops insularis* venom glands, we identified for the first time a cDNA coding for a putative Vascular Endothelial Growth Factor-like (VEGF-like) protein. The deduced primary sequence, after complete sequencing of the longest snake venom VEGF (svVEGF) cDNA, displayed similarities with vertebrate VEGFs, placental growth factors (PlGF), platelet-derived growth factor (PDGF) and with the hypotensive factor (HF) from *Vipera aspis* venom. Its cDNA was subcloned, expressed in *E. coli* with a 6X His-tag as an insoluble monomer and purified by a Ni2+-affinity chromatography after 8M urea extraction. Antiserum against the recombinant svVEGF was generated in mice and tested in Western-blot against proteins from various snake venoms and cellular extracts of snake venom glands and other tissues. The mature svVEGF appears to be ubiquitous distributed throughout snake venoms but not in other cellular extracts. This result was also confirmed by Northern Blot studies of RNAs from other related *Viperidae* species and by cDNA cloning of svVEGF from *Bothrops jararaca* pitviper. The produced recombinant protein dimerizes after refolding processes and was biologically characterized, showing ability to increase vascular permeability. These results established that svVEGF is a novel and important active toxin during the early stages of bothropic snake bite envenoming and represents a new member of the VEGF family of proteins (1).

This work is supported by FAPESP, CNPq and Fundação Butantan

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Poster 1-5

cDNA CLONING OF PRECURSOR OF BRADYKININ-POTENTIATING PEPTIDES AND C-TYPE NATRIURETIC PEPTIDE FROM THAI SNAKE, *CALLOSELASMA RHODOSTOMA*.

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We isolated cDNA clones and elucidated the structure of the precursors of bradykinin-potentiating peptides (BPPs) and C-type natriuretic peptide (CNP) from various crotalinae snakes, *Bothrops jararaca*, *Agkistrodon blomhoffi*, *Trimeresurus flavoviridis* and etc.

In the present study, we have isolated cDNA coding for BPP-CNP precursor from the Thai snake, *Calloselasma rhodostoma* venom gland cDNA library. The isolated cDNA clone of 1.8 Kbp long encodes a precursor composed of 333 amino acid residues. The precursor consists of signal sequence, BPPs, intervening linker sequence and CNP, that is almost identical to those from the other crotalinae snakes. In the BPP region, seven repeats of BPP-like peptide with spacer sequence were found. Four of seven amino acid sequences of BPP-like peptide have the typical feature of BPP, i.e. glutamine residue in the amino terminus and proline residue in the carboxyl terminus. The sequence of BPP might have highly diversified as compared with those from other snakes, while the C-type natriuretic peptide found in the C-terminus was conserved between all organisms. These results suggest the wide distribution of the BPP-CNP genes among the crotalinae subfamily. The evolution of the BPP-CNP gene from crotalinae snakes would be discussed.

Poster 1-6

A NEW GENE STRUCTURE OF DISINTEGRIN FAMILY: A SUBUNIT OF DIMERIC DISINTEGRIN HAVE SHORT CODING REGION

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Disintegrin is a potent platelet aggregation inhibitor isolated from various snake venoms. The cDNA of the snake venom disintegrin family precursors is well known to encode by pre-peptide, metalloprotease, spacer and disintegrin domain. Recently, new type of disintegrins, dimeric disintegrins, were isolated and characterized. We isolated a novel heterodimeric disintegrin, acostatin, from the venom of *Agkistrodon contortrix* and both chains had the Arg-Gly-Asp (RGD) sequence for binding platelet glycoprotein IIb/IIIa complex. The cDNA of the α chain had only the length of half against the cDNA of the β chain.

The cDNA of the acostatin β chain had identity to a well-known motif of disintegrin precursors. Furthermore, another heterodimeric disintegrin, piscivostatin (1), also had the same domain structure to that of acostatin in its cDNA. These results indicate that the cDNAs of heterodimeric disintegrin subunits have quite a different length of coding region with a novel domain structure on disintegrin-family proteins.

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Poster 1-7

ISOLATION AND PARTIAL CHARACTERIZATION OF AN ANTI-PLATELET AGGREGATION AND ANTITUMORAL PROTEIN FROM *Bothrops jararacussu* SNAKE VENOM.

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Platelet aggregation is a key step related with blood clotting and snake venoms stand out as a rich source of proteins able to promote or inhibit this aggregation(1). *Bothrops jararacussu* snake venom was fractionated on a Sephadex G-75 column equilibrated and eluted with 0.05M, pH 8,0 ammonium bicarbonate buffer(2). One of the several fractions thus obtained (SIII) showing anti-platelet aggregation activity was submitted to an ion exchange chromatography on SP-Sephadex C-25 at pH 5.0 using an ammonium acetate concentration gradient from 0.1 to 2.0M. The active fraction was finally rechromatographed by HPLC where from a highly purified protein (SIIISPIIIB), able to inhibit ADP or thrombin-induced platelet aggregation was obtained, showing a single electrophoretic band both by acidic and basic PAGE. A single band was also detected by SDS-PAGE and isoeletric foccusing, corresponding to an approximate Mr of 30,000 and a pI 7.5, respectively. It's N-terminal sequence was: SVDFDDECDWPACICCAATCKL No proteolytic activity upon fibrinogen was observed but a significant antitumoral activity was detected, which was equivalent to that of methotrexate, when assayed against SK-BR-3 (human mamma carcinoma) or C-8161-1 (melanoma) cells. It's primary structure determination is in progress.

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Poster 1-8

THE cDNA SEQUENCE OF THE NON-ENZYMATIC SUBUNIT OF PSEUTARIN C, A PROTHROMBIN ACTIVATOR FROM THE BROWN SNAKE (*PSEUDONAJA TEXTILIS*) SHOWS STRUCTURAL SIMILARITY TO BLOOD COAGULATION FACTOR V

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Snake venom prothrombin activators are classified into four groups based on their cofactor requirements (1,2). Group C prothrombin activators are large (~250 kDa) protein complexes with multiple subunits (3). They are functionally similar to coagulation factor Xa [FXa] and factor Va [FVa] complex. They require Ca^{2+} ions and phospholipids for their activity. Although functional similarity between group C activators and mammalian FXa - FVa complex is known, structural details for this class of prothrombin activators are lacking. Structural data of the group C prothrombin activators will help us understand the structure-function relationship and molecular mechanisms of the formation of the prothrombinase complex. We have purified Pseutarin C, a group C activator from *Pseudonaja textilis* venom, using gel filtration and hydroxyapatite chromatography. The two subunits of pseutarin C were separated by RP-HPLC. Partial amino acid sequence of the non-enzymatic subunit was obtained after subjecting it to chemical and enzymatic digestion. Degenerate primers were designed based on the peptide sequences. Using primer-walking sequencing strategy we have determined the complete cDNA sequence of the non-enzymatic subunit. The cDNA of the non-enzymatic subunit encodes a protein of 1457 amino acids, which includes a 30-residue signal peptide, and a mature protein of 1427 amino acids. cDNA blot analysis showed a single transcript of ~ 4 kb. Analysis of the deduced amino acid sequence shows ~31% identity to mammalian factor V and by homology has similar domain architecture consisting of A1-A2-B-A3-C1 and C2 domains. Interestingly, the B domain of pseutarin C is very short as compared to mammalian FV. Our data demonstrates that the non-enzymatic subunit of group C prothrombin activators is structurally similar to mammalian FV.

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Poster 1-9

OCCURRENCE OF A NOVEL *O*-LINKED XYLOSE-GLCNAC DISACCHARIDE IN TROCARIN, A FACTOR XA HOMOLOG FROM SNAKE VENOM

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Trocarin is a 46515-Dalton group D prothrombin activating glycoprotein from the venom of the Australian elapid, *Tropidechis carinatus*. Amino acid sequencing and functional characterization of trocarin demonstrated that it is a structural and functional homolog of mammalian blood coagulation factor Xa. In this study we show that, in contrast to mammalian Xa, which is not glycosylated, trocarin contains one *O*-linked carbohydrate moiety in its light chain and one *N*-linked carbohydrate oligosaccharide in its heavy chain. Mass spectrometry and sugar compositional analysis indicate that the *O*-linked carbohydrate moiety is a mixture of Xyl-GlcNAc- and GlcNAc- linked to Ser 52. These O-linked carbohydrate moieties appear to be the products of novel Golgi glycosyltransferases. Thus, trocarin is the first known secretory glycoprotein glycosylated in the Golgi with *O*-linked GlcNAc. The *N*-linked carbohydrate of the heavy chain is a sialylated, diantennary oligosaccharide that is located at the lip of the active site of the prothrombin activator.

Poster 1-10

PURIFICATION AND CHARACTERIZATION OF A FIBRIN(OGEN)OLYTIC KININOGENASE FROM THE VENOM OF *TRIMERESURUS JERDONNI* SNAKE

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A novel kininogenase was purified from the venom of *Trimeresurus jerdonni* snake by a combination of DEAE Sephadex A-50 anion-exchange chromatography, Sephadex G-75 gel filtration and reversed-phage high performance liquid chromagraphy(RP-HPLC). It is a single chain protein with a molecular weight of 28000 Da in non-reduced condition and 32000 Da in reduced condition respectively. It can degrade Aα, Bβ chain of human fibrinogen slowly. Unlike other kininogenase from other snake venom, it show higher preferential hydrolysis to S-2228 than S-2266 and S-2302. Its enzymatic activity was inhibited by PMSF, not by EDTA, indicating that it is a serine proteinase. N-terminal sequence is also similar to other snake venom serine proteinase.

Poster 1-11

PURIFICATION AND CHARACTERIZATION OF JERDOFIBRASE, A SERINE PROTEASE FROM THE VENOM OF *TRIMERESURUS JERDONII* SNAKE

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A fibrin(ogen)olytic serine protease from *Trimeresurus jerdonii* venom was identified and purified to SDS-polyacrylamide gel electrophoresis homogeneity. It is a single chain polypeptide with a molecular weight of 32kDa under reduced condition and 28kDa under non-reduced condition, respectively. The venom protease catalyzed the hydrolysis of some chromogenic substrates such as S2238, S2160, S2302 and S2251. It degraded B β -chain of human fibrinogen preferentially. Also the enzyme degraded fibrin directly. Its enzymatic activity was completely inhibited by phenylmethylsulfonyl fluoride (PMSF), but not affected by EDTA. That suggested it was a serine protease. N-terminal sequence of the purified component showed high homology with other snake venom serine proteases.

Poster 1-12

BIOCHEMICAL CHARACTERIZATION OF JERDOFIBRASE-2, AN ALPHA-FIBRINOGENASE WITH HIGH MOLECULAR WEIGHT ISOLATED FROM THE VENOM OF *TRIMERESURUS JERDONII*

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A fibrin(ogen)olytic enzyme with high molecular weight named jerdofibrase-2 was purified from the venom of *Trimeresurus jerdonii* by the multiple-step chromatography including DEAE Sephadex A-50 anion-exchange chromatography, Sephadex G-100 (superfine) gel filtration and reversed-phase high performance liquid chromatography (HPLC). SDS-PAGE shows that the enzyme consists of a single polypeptide chain with an approximate molecular weight of 55 kD. It is a glycoprotein containing 35.8% neutral carbohydrate. The N-terminal amino acid sequence of the jerdofibrase-2 has great homology with other serine proteases from snake venoms. The enzyme catalyzed the hydrolysis of some chromogenic substrates such as S-2238 and S-2302. It degraded A α-chain of human fibrinogen preferentially. Also, the enzyme degraded fibrin directly. The enzyme activity was completely inhibited by phenylmethylsulfonyl fluoride (PMSF), soybean trypsin inhibitor, but not affected by EDTA, indicating it a serine protease. In addition, disulfide bridge is important for its activity, since it's activity was partly inhibited by DTT and *l*-cysteine.

Poster 1-13

PURIFICATION AND CLONING OF A NOVEL C-TYPE LECTIN-LIKE PROTEIN WITH PLATELET AGGREGATION ACTIVITY FROM *TRIMERESURUS MUCROSQUAMATUS* VENOM

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TMVA, a novel C-type lectin-like protein that induces platelet aggregation in a dose-dependent manner, was purified from the venom of *Trimeresurus mucrosquamatus*. It consists of two subunits, α (15536 Da) and β (14873 Da). The mature amino acid sequences of the α (135 amino acids) and β subunits (123 amino acids) were deduced from cloned cDNAs. Both of the sequences show great similarity to C-type lectin-like venom proteins, including a carbohydrate recognition domain (CRD). The cysteine residues of TMVA are conserved at positions corresponding to those of flavocetin-A and convulxin, including the additional Cys135 in the α subunit and Cys3 in the β subunit. SDS-PAGE, mass spectrometry analysis and amino acid sequence showed that native TMVA exists as two convertible isoforms of $(\alpha\beta)_2$ and $(\alpha\beta)_4$ with molecular weights of 63680 Da and 128518 Da. The $(\alpha\beta)_2$ complex is stabilized by an interchain disulfide bridge between the two αβ-heterodimers, whereas the stabilization of the $(\alpha\beta)_4$ complex seems to involve non-covalent interactions between the $(\alpha\beta)_2$ di-dimers.

Poster 1-14

CHARACTERIZATION AND CLONING OF A NOVEL PHOSPHOLIPASE A_2 FROM THE VENOM OF *TRIMERESURUS JERDONII* SNAKE

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A phospholipase A_2 (PLA_2), called jerdoxin, was isolated from *Trimeresurus jerdonni* snake venom and partially characterized. The protein was purified by three chromatographic steps. SDS-PAGE in the presence or absence of dithiothreitol showed that it had a molecular mass of 15 kDa. Jerdoxin had an enzymatic activity of 39.4 μmoles/min/mg towards egg yolk phosphatidyl choline (PC). It induced mild edema in the footpads of mice. In addiction, jerdoxin exhibited indirect hemolytic activity. About 97% hemolysis was observed when 2 μg/ml enzyme was incubated for 90 min in the present of phosphatidyl choline (PC) and Ca^{2+}. No detectable hemolysis happened when PC was not added. Ca^{2+} was necessary for jerdoxin to exert its hemolytic activity, since only 52% hemolysis was seen when Ca^{2+} was absence in the reaction mixture. Furthermore, jerdoxin inhibited ADP induced rabbit platelet aggregation and the inhibition was dose dependent with an IC_{50} of 1.0 μM. The complete amino acid sequence of jerdoxin deduced from cDNA sequence shared high homology with other snake venom PLA_2s, especially the D49 PLA_2s. Also, the residues concerned to Ca^{2+} binding are conserved. This is the first report of cDNA sequence of *Trimeresurus jerdonii* venom PLA_2.

Poster 1-15

AMINO ACID SEQUENCE AND TOXICOLOGICAL PROPERTIES OF A PHOSPHOLIPASE A_2 ENZYME FROM *HOPLOCEPHALUS STEPHENSI* SNAKE VENOM

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Stephen's banded snake (*Hoplocephalus stephensii*) is an elapid commonly found in south Queensland and north eastern New South Wales. We recently purified a phospholipase A_2 (PLA_2) enzyme, HS-PLA_2, from the venom of *H. stephensii* by a single-step purification method using a reverse phase column. The molecular mass was 14122. The amino acid sequence of HS-PLA_2 was determined by sequencing peptides of reduced and pyridylethylated protein generated by endoprotenase Lys-C. Mice injected with HS-PLA_2 (intraperitoneal injections; 30mg/Kg) appeared to be ill and remained motionless even when handled. It was interesting to note that their abdominal area were soft upon touch, seemingly indicative of loss in muscle tone. Histological examination of all vital organs (spleen, kidney, lung and liver) of mice injected with HS-PLA_2 (intraperitoneal injections; 100 mg/kg) indicated they had severe tissue damage. We are currently investigating the mechanism of various tissue damage induced by HS-PLA_2.

Poster 1-16

AN INFLAMMATORY AGENT, VENOM PHOSPHOLIPASE A_2, MODULATES THE EXPRESSION OF LUNG AQUAPORINS

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Aquaporins (AQPs) are water channels found ubiquitously in mammals, plants and bacteria. These are a family of proteins that form pores in cell membranes, allowing water to move in or out of the cells. To date, 11 mammalian aquaporins have been identified, some of which are permeable only to water whilst others allow small solutes and ions to pass through. Though their physiological functions are not fully understood, they have been implicated in a number of pathophysiological conditions such as congestive heart failure, acute respiratory distress syndrome, infection, lung injury, oedema, brain tumour and other neurological disorders. Studies to elucidate the roles aquaporins play in water homeostasis in the body have been hampered by the lack of modulators of gene expression. Mercury chloride is the only known inhibitor of aquaporins. However, its toxicity prevents it from being used *in vivo*. A candidate modulator of aquaporin expression is venom secretory phospholipase A_2 (PLA_2). $sPLA_2$ induces inflammation, which is characterized by tissue injury and oedema. We have shown that intratracheal injection of $sPLA_2$ from the Malayan spitting cobra, *Naja sputatrix*, at a dose equivalent to its LD_{50} value, causes pulmonary oedema and infiltration of neutrophils in rat lungs after 3 hrs. In contrast, intravenous injection of the same dose of sPLA2 did not produce visible inflammation in the lungs. However, realtime PCR analysis indicated an upregulation of expression of AQP 1, 3, 4 and 5 genes.

Poster 1-17

VENOM NERVE GROWTH FACTOR AS A MODULATOR OF AQUAPORINS IN THE BRAIN

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Nerve growth factor (NGF) is a polypeptide that supports neuronal maintenance and survival. Extension of neurites have been observed upon addition of NGF to Pheochromocytoma (PC12) cells. We have recently cloned and expressed cDNAs encoding the venom nerve growth factor (vNGF) in *Naja sputatrix*. vNGF (14kDa) is produced from a precursor protein of 23 kDa. This vNGF has also been found to elicit neurite outgrowth in PC12 cells. However, it has been found to provide an additional protective effect to neuronal cells (derived from hippocampus) by down-regulating the expression of Aquaporin 4 and up-regulating the expression of Aquaporins 1 and 9 as well as the inwardly rectifying potassium channel (Kir 4.1) genes.

Poster 1-18

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A PHOSPHOLIPASE A_2 MYOTOXIN INHIBITOR FROM *BOTHROPS MOOJENI* SNAKE PLASMA

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A protein was isolated from *Bothrops moojeni* snake plasma by affinity chromatography using immobilized myotoxins on Sepharose gel. It was able to neutralize the enzymatic, toxic and pharmacological activities of different basic phospholipases A_2 homologues from the venoms of *B. moojeni*, *B. pirajai* and *B. jararacussu*. Biochemical characterization of this myotoxin inhibitor protein (BmjMIP) showed it to be an oligomeric glycoprotein with a Mr of 23,000 to 25,000 for the monomeric subunit. BmjMIP was stable in the pH range from 4.0 to 12.0, as well as between 4°C and 80°C, and even after deglycosilation. The role of the carbohydrate moiety was investigated and found not to affect the *in vitro* function of the inhibitor. The corresponding cDNA obtained by RT-PCR from the liver of this snake has 500 pb which codify for a mature protein of 147 and a signaling peptide of 19 amino acid residues. The primary structure of BmjMIP showed a high similarity with other snake phospholipase A_2 inhibitors (PLIs) which conserve the recognizing domain for carbohydrates (CRDs) and the glycosylation site (Asn103). The functional characterization showed that BmjMIP has a wide range of inhibitory properties of basic PLA_2s from *Bothrops* venoms (anti-enzymatic, anti-myotoxic, anti-edema inducing, anti-cytotoxic, anti-bactericidal and anti-lethal). In spite of that, this inhibitor was little efficient to neutralize the biological activities of crotoxin B, the PLA_2 homologue associated with crotapotin in the *Crotalus durissus terrificus* snake venom.

FINANCIAL SUPPORT: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Poster 1-19

MICRURUS PHYRROCRYPTUS ("MBOI-CHUMBÉ-GUAZÚ") VENOM PRODUCES MYOTOXICITY IN RATS AND MICE.

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The accidents caused by *Micrurus* (coral snake) in Argentina are not very frequent (less than 1%). The *Micrurus* bite constitutes a medical urgency that requires urgent administration of antivenom and medical care, frequently in an intensive care unit. In the country have been described *Micurus* (*M.*) *frontalis*, *M. altirrostris*, *M. mesopotamicus* (or *baliocoryphus*) *M. phyrrocryptus*, *M. corallinus* and *M. lemniscatus.* This venoms showed a high lethal potency. The i.p. LD_{50} (µg/g, CF-1 mice, 20g) are 1.3 ± 0.3µg for *M. corallinus* and *M. phyrrocryptus* and 0.5 ± 0.2µg for *M. mesopotamicus.* The *Micrurus* widest distributed (from the Southern to the Northern provinces) is *M. phyrrocriptus*, and possibly this is the principal responsible of envenomation by *Micrurus* in Argentina. Although the envenomation by *Micrurus* is clearly neurotoxic, in the assays for determination of lethality or in the neutralization assays, the occurrence of dark urine is a frequent finding. As myotoxicity was described in the venoms from *Micrurus* species of Central America (1) and South America (2), we injected via intramuscular rats (Whistar, 250 g) and mice (CF-1, 20g) with 10 - 100 µg of *M. phyrrocryptus* venom to test its possible action on skeletal muscle. Samples of blood were taken to determine the CK level 2, 24, 48 and 72 hs post inoculation. Animals were sacrificed 24, 48 and 72 hs post inoculation and samples were fixed in 10% formaldehyde in 0.15 M NaCl, 0.1 M phosphate buffer pH 7.0. Samples were included in paraffin, sliced, mounted on microscope slides and stained with hematoxylin-eosin for microscopic examination. Differences in CK levels ($p<0.05$) were observed in inoculated animals when compared with controls injected with NaCl 0.15M. The histopathological examination showed light muscle inflamation with inflamatory infiltrate, edema and necrosis in 24 hs samples. Samples of 48 and 72 hs showed very important extensive muscular inflamation and necrosis with an important polymorphonuclear infiltrate. This findings showed that the venom of *M. phyrrocryptus* may produce myotoxicity in experimental animals. Studies are carrying to determine the components involved in the myotoxicity and tissue damage caused by this venom.

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Poster 1-20

CLONING AND CHARACTERIZATION OF NOVEL SNAKE VENOM PROTEINS, WHICH BLOCK SMOOTH MUSCLE CONTRACTION

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Over the past 30 years, a plethora of toxins have been isolated from poisonous organisms, such as snakes, scorpions, spiders, and microorganisms. In this study, we isolated a 25-kDa novel snake venom protein, designated ablomin, from the venom of the Japanese Mamushi snake (*Agkistrodon blomhoffi*) (1). The amino acid sequence of this protein was determined by peptide sequencing and cDNA cloning. The deduced sequence showed high similarity to helothermine from the Mexican beaded lizard (*Heloderma horridum horridum*), which blocks voltage-gated calcium and potassium channels, and ryanodine receptors. Ablomin blocked contraction of rat tail arterial smooth muscle elicited by high K^+-induced depolarization in the 0.1-1 μM range, but did not block caffeine-stimulated contraction. Furthermore, we also isolated three other proteins from snake venoms that are homologous to ablomin and cloned the corresponding cDNAs. Two of these homologous proteins, triflin and latisemin, also inhibited high K^+-induced contraction of the artery. These results indicate that several snake venoms contain novel proteins with neurotoxin-like activity.

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Poster 1-21

PHARMACOLOGICAL CHARACTERIZATION OF CONTRACTION INDUCED BY BLOMHOTIN, A NOVEL PEPTIDE FROM THE VENOM OF *AGKISTRODON HALYS BLOMHOFFII*, IN RAT FUNDUS

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Blomhotin (BH) is an 11-amino acid peptide (pGlu-Gly-Arg-Pro-Pro-Gly-Pro-Pro-Ile-Pro-Arg) discovered in the venom of the Japanese mamushi, *Agkistrodon halys blomhoffii* (1). Blomhotin exhibits potent contractile activity on rat stomach fundus. In this study, we investigated the role of calcium ion in the blomhotin-induced contraction and the structure-activity relationship using fragments and analogues of blomhotin. Moreover, a candidate of target molecule of blomhotin was investigated.

1) Removal of extracellular Ca^{2+} completely abolished maximum blomhotin-induced contractile responses. The contraction was not inhibited by nifedipine or varapamil, selective blockers of voltage-operated Ca^{2+} channel (VOCs), but was inhibited by SK&F96365, a nonselective blocker of receptor-operated Ca^{2+} channels (ROCs) and VOCs.

2) We carried out a structure-activity study of blomhotin and its related peptides, and the findings suggested that the C-terminal portion of blomhotin, Pro-Ile-Pro-Arg, is responsible for the full activation of the blomhotin receptor in rat stomach fundus.

3) Cross-desensitization between blomhotin and anaphylatoxin C3a was observed and the contraction induced by blomhotin was inhibited by [fmoc-Glu^{70}, $Ala^{71,72}$, Lys^{74}]C3a(70-77), an antagonist of C3a.

In conclusion, blomhotin-induced contraction of rat fundus smooth muscle is mediated by a C3a receptor which couples to the influx of extracellular calcium via ROCs.

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Poster 1-22

BLOMHOTIN-RELATED PEPTIDES INCREASE VASCULAR PERMEABILITY THROUGH MAST CELL ACTIVATION

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Blomhotin (BH) is a cationic peptide (pGlu-Gly-Arg-Pro-Pro-Gly-Pro-Pro-Ile-Pro-Arg) discovered in the venom of the Japanese mamushi, *Agkistrodon halys blomhoffii* (1). Many basic vasoactive peptides including bradykinin and substance P have been reported to induce increase of vascular permeability. Present study was undertaken to investigate ability of blomhotin-related peptides to induce increase of vascular permeability and histamine release from mast cells.

1) The intradermal injection of blomhotin produced a dose-dependent extravasion of Pontamine sky blue dye in rat skin. Blomhotin was less active than bradykinin. Interestingly, removal of pGlu from the N-terminus brought about a marked increase in activity which is comparable to bradykinin. Deletion of three amino acids from the N-terminus (producing BH(4-11)) caused quite a loss of activity. Removal of Arg from the C-terminus caused decrease of activity. These results suggest that basic amino acids may be important like other peptides such as bradykinin, substance P and neurotensin.

2) Effect of BH(2-11) on histamine release in purified rat peritoneal mast cells manifested a bell-shaped dose response curve. The peak effect was observed at the concentration of 100•M. A similar response was observed using [Phe^{9}]BH(2-11) as a stimulant. [Phe^{9}]BH(2-11) was more potent than BH(2-11). In contrast, bradykinin induced histamine release in a concentration-dependent manner up to 1000•M.

3) Pretreatment of mast cells with pertussis toxin blocked histamine release induced by [Phe^{9}]BH(2-11), implicating involvement of pertussis toxin-sensitive G protein.

In conclusion, BH(2-11) and [Phe^{9}]BH(2-11) induced increase of vascular permeability through mast cell activation. The peptides exhibited inhibitory effects on mast cell activation in high concentration. The inhibitory action of the blomhotin-related peptides is unique in contrast to the other basic secretagogues. The blomhotin-related peptides may become useful tools to study the mechanism of mast cell activation.

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Poster 1-23

A 17-MER PHOSPHOLIPASE A_2-INHIBITORY PEPTIDE NEUTRALIZES THE TOXICITY OF CANDOXIN, A NON-CONVENTIONAL (WEAK) TOXIN FROM *BUNGARUS CANDIDUS*

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A 17-mer peptide P-NT.II derived from the primary structure of PIP, an endogenous phospholipase A_2 inhibitor from *Python reticulatus* serum, shows strong inhibitory activity against secretory phospholipase A_2. P-NT.II inhibits enzyme activities of $sPLA_2$s from snake venoms and human non-pancreatic secretory PLA_2 from synovial fluid of patients with arthritis. Interestingly, besides its PLA_2 inhibitory activity, P-NT.II appears to neutralize the *in vivo* toxicity of candoxin, a novel non-conventional (weak) toxin from the Malayan krait *Bungarus candidus* when the toxin was pre-incubated with the peptide prior to intraperitoneal injection into albino mice. In contrast, P.NT.II failed to neutralize the toxicity of potent α-neurotoxins such as erabutoxin-b, α-bungarotoxin, or α-cobratoxin, under similar experimental conditions. The effects of P-NT.II on the blockade of nerve-evoked twitch responses produced by candoxin (20 μg/ml) or erabutoxin-b (2 μg/ml) in the mouse hemidiaphragm were also studied. P-NT.II produced significant inhibition ($p< 0.05$) of the twitch blockade induced by candoxin at lower peptide concentrations (6.7 μg/ml) whereas it weakly inhibited the erabutoxin-b induced twitch blockade at a relatively higher concentration (13.4 μg/ml). The experimental evidence for the direct and specific binding of the active biotinylated-P-NT.II to candoxin and other neurotoxins was also provided by ELISA. Our preliminary data suggests that P-NT.II appears to neutralize the in vivo effects of candoxin in mice and in addition, partially inhibit the twitch blockade produced by candoxin in isolated nerve-muscle preparations. Synthetic peptides like P-NT.II that bind neurotoxins may yield competitive inhibitors of neurotoxin binding to acetylcholine receptor, and could well serve as potential lead compounds for effective antidotes against snake envenomation.

Poster 1-24

PUTATIVE FUNCTIONAL DETERMINANTS OF CANDOXIN-INDUCED BLOCKADE OF MUSCLE (αβγδ) AND NEURONAL α7 NICOTINIC ACETYLCHOLINE RECEPTORS

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We have recently isolated and purified candoxin, a novel non-conventional toxin from the Malayan krait *Bungarus candidus* (1). Its NMR structure revealed a three-finger scaffold with four conserved disulfide bridges and an additional (5^{th}) disulfide bridge located at the tip of the N-terminal loop (loop I). Nanomolar concentrations of candoxin produced functional block of acetylcholine-evoked currents in oocyte-expressed rat muscle (αβγδ) (IC_{50} ~10 nM) as well as rat neuronal α7 (IC_{50} ~50 nM) nicotinic acetylcholine receptors (1). Interestingly, candoxin lacks the helix-like segment cyclized by the fifth disulfide bridge at the tip of the middle loop (loop II) of long-chain α/κ-neurotoxins, reported to be crucial for binding to α7 receptors (2). Nevertheless, its NMR structure showed the presence of some functionally invariant residues involved in the interaction of short-chain α-neurotoxins with muscle (αβγδ) and long-chain α/κ-neurotoxins with both, muscle and α7 receptors. Moreover, there was remarkable similarity in the spatial disposition and orientation of these critical residues in candoxin and the short-chain α-neurotoxin, erabutoxin-a and the long-chain α/κ-neurotoxin, α-cobratoxin. Non-conventional or weak toxins are generally known to inhibit muscle (αβγδ) receptors with poor affinity, in micromolar concentrations at best (3). Clearly therefore, candoxin is a novel toxin that antagonizes both nicotinic receptor subsets in low nanomolar concentrations. In addition, candoxin possibly utilizes additional functional determinants that assist in the recognition of neuronal α7 receptors and compensate for the absence of the critical Cys-Cys conformation at the tip of loop II present in long-chain α/κ-neurotoxins.

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Poster 1-25

TWO SPECIES OF COBRAS DISTRIBUTED IN THE MAINLAND OF CHINA CONTAIN SIMILAR SHORT-CHAIN NEUROTOXINS

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There are two main cobras in the mainland of China, one is *Naja kaouthia* mainly distributed in the southwest of China. The other is *Naja atra* distributed in the east and south of China. Two short-chain neurotoxins were purified and sequenced from the venoms of *Naja atra* (in zhejiang province), Which showed the same amino acid sequence to Cobrotoxin and Cobrotoxin b from the same specie but distributed in Taiwan. Three short-chain neurotoxins named as NT-I, NT-II, NT-III were also purified, sequenced and cloned from the venom of *naja kaouthia* (in Yunnan province, near to Thailand). NT-I, NT-III have the same amino acid and nucleotide sequence to Cobrotoxin and Cobrotoxin-b, respectively. NT-II is a novel one, which consisted of 61 amino acids, and only two residues difference compared with NT-III. Cobratoxin, one long-chain neurotoxin and another 62 amino acid short-chain neurotoxin, instead of NT-I, NT-II, NT-III were reported to mainly exist in the same species in Thailand. To date, Cobratoxin were also not found in the *Naja kaouthia* venom in China. These founding indicates that there exist other factor to determine the component of snake venom besides species and geographic location.

Poster 1-26

AVIDIN-BIOTIN OPTICAL IMMUNOASSAY FOR THE DETECTION OF *TRIMERESORUS POPEORUM* VENOM

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After successful on development of avidin-biotin optical immunoassay (AB-OIA) for the detection of single snake toxin (1), AB-OIA has been adapted for the detection of whole venom of green pit viper (*Trimeresorus popeorum*) in human blood, plasma and urine. Species-specific antibody against *T. popeorum* was prepared from hyper-immunized rabbit by affinity purification. This species-specific was then immobilized on an optically active silicon surface (SILIAS™ wafer). The test samples were applied on that surface and the antigen antibody reaction was monitored by the addition of biotinylated species-specific antibody, avidin-horseradish peroxidase conjugate and tetramethyl-benzidine substrate. This AB-OIA is simple, requires only 40 µL of biological fluid and can be performed without specialized equipment. The assay was specific for *T. popeorum* and could detect venom levels as low as 0.05 ng/mL of sample buffer and 0.1 ng/mL of whole blood or plasma within 25 minutes. The study on experimental envenomation of *T. popeorum* venom in rats showed that this AB-OIA is effective in detection of *T. popeorum* venom. The silicon assay surface technology enables us to directly visualize a physical change in the thickness of the optical layer coated on the top of it. This change is due to the specific binding of venom antigens and antivenom antibodies and when the substrate is added, this binding event is amplified. This change in the thickness alters the reflected light path and is visualized as a colour change. Further studies to incorporate different AB-OIA into snake species identification kit are in progress.

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Poster 1-27

THE INTRAPERITONEAL TOXICITY IN MICE OF A RANGE OF AUSTRALIAN AND EXOTIC SNAKE VENOMS

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Snake venom and antivenom research often requires, and may be driven by, an understanding of relative toxicities and mechanisms of venom lethality. A standard method of comparing overall venom toxicity is 'lethal dose' testing. To date only one comprehensive and statistically sound study of the lethality of Australian elapid venoms has been undertaken[1]. That study examined the toxicity of a range of Australian and exotic snake venoms after subcutaneous injection (s.c.) through the calculation of the lethal dose for 50% of animals (LD50). An outcome of that investigation was a rank order of lethality with the 'most dangerous snake' being listed as the inland taipan (*Oxyuranus microlepidotus*). We present new data on the lethality of a range of Australian and exotic venoms snakes, after intraperitoneal injection (i.p.), using a modification of the LD50 test. Freeze-dried venoms were reconstituted with 0.9% saline with 0.1% bovine serum albumin and injected intraperitoneally into SPF IMVS Balb/c 28±2 gram male mice. Mice were continually and closely observed for a full eight hour period with clinical signs of envenomation being assessed during that time. The results of this modified intraperitoneal LD50 test were calculated for 30 species including exotic elapids (eg *Bungarus fasciatus* [West Java] and *Naja mossambica* [Africa]) as well as vipers (*Bitis nascicornus* [Africa]) and crotalids (*Agkistrodon bilineatus* [Mexico]). Three prothrombin activators from Australian brown, tiger and taipan species were also examined. Amongst these venoms, this study provides the first formal animal toxicity data regarding the venoms from *Pseudechis butleri*, *Oxyuranus scutellatus canni* and *Micropechis ikaheka.* Considering the results, the venoms could be broadly characterised as those that had near identical i.p. and s.c. LD50 values and those that were dissimilar - either increased or decreased. Examples include the common or mainland tiger snake (Victoria) *Notechis scutatus* (s.c. LD50 = i.p. LD50 =0.12 mg/kg), Island tiger snake (SA) *Notechis ater niger* (s.c. LD50 = 0.099 mg/kg but i.p. LD50 =0.40 mg/kg), and the western brown or Gwardar (Perth) *Pseudonaja nuchalis* (s.c. LD50 = 0.338 mg/kg but i.p. LD50 =0.035 mg/kg). Due to these differences, the rank order of toxicity observed through the i.p. route of administration is somewhat modified from that of s.c. administration. The top five by i.p. administration are (in decreasing order of potency) the inland taipan, PNG taipan, coastal taipan, the gwardar and the eastern brown.

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Poster 1-28

INFLUENCES ON VENOM YIELD IN AUSTRALIAN TIGERSNAKES (*NOTECHIS SCUTATUS*) AND BROWNSNAKES (*PSEUDONAJA TEXTILIS: ELAPIDAE, SERPENTES)*

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The rates at which venomous animals produce toxins are of obvious biological and medical importance, but factors influencing those rates remain poorly understood. We gathered data on venom yield (wet mass of venom) and percentage solids (dry mass of the venom divided by wet mass) for 53 eastern brown snakes (*Pseudonaja textilis*) and 36 mainland tiger snakes (*Notechis scutatus*) over a four-year period at Venom Supplies Pty Ltd, a commercial venom-production facility in South Australia. Tiger snakes produced about threefold more venom (by wet mass) than brown snakes, but with slightly lower percentage solids. Both species showed significant geographic variation in percentage solids. Venom yields varied as a function of the snake's sex and geographic origin, but these effects were secondary consequences of geographic and sex-based differences in body size. Relative head size affected venom yield in brown snakes but not tiger snakes. Overall, the amount of venom that a snake produced during milking was affected by its species, its geographic origin, its body size and relative head size, and by the time of year that it was milked, as well as by interactions among these factors. Body size was the most important effect on venom yield, with yields increasing more rapidly with size in brown snakes than in tiger snakes. Research at the intersection of snake ecology and venom characteristics has great potential, but will require a genuinely interdisciplinary approach.

Poster 1-29

DEVELOPMENT OF A GLOBAL INTERNET RESOURCE FOR CLINICAL TOXINOLOGY

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We are developing an internet-based knowledge system for clinical toxinology, globally focussed. The project is based on acquisition and incorporation of information from the global literature and from clinical experience. Information is stored principally in a series of linked databases. These form the basis for serving searchable information to the internet. The areas we aim to cover include snakes, other major terrestrial venomous vertebrates, scorpions, spiders, other major venomous terrestrial invertebrates, marine venomous vertebrates and invertebrates, marine poisonous vertebrates and invertebrates, poisonous plants and poisonous mushrooms. For all venomous organisms, the data is stored as individual species or subspecies records, therefore taxonomy is a vital part of our information system. As time and resources permit, we will progressively add new records and upgrade existing records to include major taxonomic detail. For all venomous snakes this detail is already present in the databases. In addition to taxonomic information, we will include, where relevant and available, basic biologic information, geographic distribution, including a distribution map, detail of venom and components, clinical information, summaries of published case reports, references, treatment advice, including first aid, available antivenoms and details of antivenom producers. For each species, this information will vary from 3 A4 pages to >100 pages, depending on how much is known about that organism. It will be possible to search based on common name, family, genus, species, country, region, taxonomic features (snakes only), diagnostic features (a form of expert system), antivenom name, antivenoms for each country, antivenoms for specific organisms, first aid. In addition to this dynamic data served from databases, there will be "static" pages containing more general information, not related to a particular species. The system will allow us to update information weekly. To sustain this massive project, after expiry of initial funding, we will have a subscription system. Detailed information will only be available to subscribers. Basic information, including first aid, will be available free to everyone. Subscriptions will support ongoing maintenance and development of the site, particularly funding staff required to update data. Subscribes will also be able to contribute to the site by submitting detailed case reports. These will be used to update clinical information. The site address will be http://www.toxinology.com.

Poster 1-30

ANTIVENOM USE IN AUSTRALIA; REPORTS OF USE 1994-2002

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All antivenom vials produced by CSL Ltd in Australia have a "Report of Use" form included and doctors using antivenom (AV) are encouraged to fill these in and return them to CSL. This has been the major method of assessing significant envenoming epidemiology in Australia. It is clearly a flawed method, but there is no legal requirement for forms to be completed and returned. It is estimated that only around 30% of occasions of use of antivenom result in submission of a form. Since late 1994 we have entered data from returned forms into a database. Currently this database holds 2807 reports, from November 1994 to January 2002. 94.7% of reports are from Australia, the remainder being from overseas or location unidentified. This highlights a major problem with the reports; highly variable quality of information. Most common Australian states for reports, from most to least common, were Queensland, Western Australia, New South Wales, Victoria, South Australia, Northern Territory, Tasmania and the ACT. Red Back spider AV was the most commonly used AV, with 1926 reports. Snakebite accounted for 371 reports, the most common snakes being brown snakes (35.6%), tiger snakes (19.1%), black snakes (7%), death adders (5.9%), and taipans (3.8%), with the snake being unknown in 22.6% of reports. Similar percentages were seen in venom detection results. There were only 7 reports of sea snake bites during the study period, 5 from Queensland (4 in fisherman on trawling boats, one from Yorkeys Knob, swimming on a beach), one each from Western Australia and the Northern Territory (NT), both offshore. There were only 10 reports f Box Jellyfish AV use, all but one in children, 6 from Queensland, 4 from the NT. There was one fatality from the NT. There were 73 reports of stonefish AV use, mostly from Queensland (56), but also from a number of overseas locations. In the 2 cases where it was reported to be of little benefit, the identity of the fish was uncertain. In most cases only 1 ampoule was required (59) and in only one report was >2 ampoules used. Overall the antivenoms appeared to be safe. No adverse reaction was reported in 98.2% of cases (2213) where this information was clear. This may underestimate reactions, because delayed (serum sickness) reactions might be missed. In only 25 reports was a major reaction noted (hypotension, bronchospasm or anaphylaxis). In many of these (9), adrenaline premedication had been used, but failed to prevent a reaction. It is unknown whether adrenaline premedication reduced the severity of the reaction. It appears that use of premedication with antivenom is not common in Australia.

Poster 1-31

SUSPECTED SNAKE-BITE : A PROSPECTIVE STUDY OF PRESENTATIONS TO AN EMERGENCY DEPARTMENT IN TROPICAL AUSTRALIA

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Snakebite is an uncommon but potentially life-threatening condition in Australia. The more common clinical scenario is of suspected snake-bite, which needs to be managed appropriately until proven otherwise. Few emergency departments see large numbers of definite snake-bites making research difficult. We prospectively studied suspected snakebites presenting to Royal Darwin Hospital, a tertiary hospital serving a large rural region in tropical northern Australia.

70 suspected snake-bites presented during the on year study period. Of these 70, there were 45 definite bites: 3 severe envenomations (2 western brown snakes [*Pseudonaja nuchalis*] and 1 mulga snake [*Pseudechis australis*]), 7 mild/moderate envenomations (1 definite and 5 probable whip snakes, 1 northern secretive snake), two non-envenomations by identified *P. nuchalis*, 5 bites by identified non-venomous snakes (Slatey grey [2] and pythons [3]) and 28 definite bites without envenomation. The remaining 25 cases were either suspected bites (8), unlikely bites (15) (8 consistent with arthropod bites) and 2 people hit by snakes. Mean patient age was 29 years (SD 19; range 2-78 yr). There were 25 females and 45 males. Definite snake-bites occurred throughout the year, peaking in May and December. In most cases (65) the snake-bite was accidental, but 3 cases occurred while catching the snake. 46 (66%) occurred on the distal extremity, and 21 occurred on proximal limbs (30%). There were 3 severe envenomations (mainly with coagulopathy), requiring antivenom treatment, 6 cases consistent with whip snake bite causing significant pain and swelling, but no deaths or major complications. Most patients had appropriate investigations; WBCT (87%), urinalysis (79%), ECG (94%) and at least two sets of bloods (63%). Of 47 VDK swabs collected: 34 not tested, 9 venom not detected (with suspicion of envenomation), 3 positive in the severe envenomations, one false positive tiger snake. Median length of stay was 17 hr (Interquartile range: 8 – 24).

The study demonstrates that although suspected snake-bite was common, severe envenomation occurred in less than 5% of cases. We also report moderate effects of some snakes of lesser medical importance, not previously well described. The study supports the proposition that a structured approach and admission policy of suspected snakebites leads to the appropriate management of severe envenomation, with no cases discharged early and no cases of non-envenomation treated with antivenom.

Poster 1-32

FIVE YEARS OF SNAKE ENVENOMING IN FAR NORTH QUEENSLAND

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Objective: To describe the epidemiology, clinical features, treatment and outcomes of patients who presented to a Cairns Base Hospital between 1996 and 2000, following elapid snake envenomation.

Method: A descriptive retrospective review of patient's records.

Results: 264 patients were admitted either to the Observation Ward in the Emergency Department, or to the Intensive Care Unit, with a diagnosis of Snakebite. 28 (10.6%) patients were envenomed, including seven children. All envenomed patients had been bitten on a limb, and 23 (82.1%) were bitten in the afternoon or evening. Twenty two (78.6%) of those envenomed required aeromedical transfer to Cairns. Only two envenomed patients had correct first aid applied immediately after the snakebite. Only one envenomed patient was asymptomatic. Bitesite Venom Detection Kits were used in 23 patients with 14 (60.9%) being positive, whereas only 3 (18%) of 17 urine Venom Detection Kits were positive. There was three false positive Venom Detection Kit results. Antivenom (AV) was administered to 20 (7.6%) patients. Sixteen patients were admitted to the Intensive Care Unit, six requiring ventilation. Five were successfully managed in the Emergency Department Observation Ward. Four patients envenomed by a taipan discharged with ongoing neurological symptoms. Five brown snake envenomations required an average of 8 ampoules of antivenom to reverse the coagulopathy. There was one death secondary to brown snake envenomation.

Conclusions: The incidence of snakebite and envenoming in far north Queensland appears higher than reported from hospitals in capital cities. First aid treatment for these north Queensland patients was poor. Uncomplicated snake envenomations can be safely managed in an Emergency Department Observation ward . Antivenom usage for brown snake envenomation was higher than currently recommended.

Poster 1-33

ANALYSIS OF INTENSIVE CARE UNIT ADMISSIONS FOR TREATMENT OF SERIOUS SNAKEBITE AT PORT MORESBY GENERAL HOSPITAL

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Snakebite remains a significant threat to public health in Papua New Guinea. As part of an ongoing study of the epidemiology of snakebite across southern rural Papua New Guinea, statistical and clinical records of admissions to the Intensive Care Unit at Port Moresby General Hospital are being analysed. Between January 1998 and December 2001 there were 283 admissions to the ICU involving seriously envenomed snakebite patients from across Papua New Guinea, the majority of whom were intubated and ventilated while in the Unit. From September to March the average number of admissions to the Unit was 6.9, while from April to August the average fell to 4.5 per month.

There were 27 deaths in the series, representing a case fatality rate of 9.54%. Complications arising from tracheotomy and/or ventilation are cited as the cause of death in 3 cases, while 2 are attributed to respiratory arrest and 2 others to cerebral haemorrhage. While the overall ratio of male: female patients was 2.21:1, males are disproportionately represented among young adults between 15-29 years where the ratio was 4.07:1. 42.94% (73/170) of male patients and 51.95% (40/77) of female patients were less than 15 years of age. The mean length of stay within the Unit was 5.5 days (range=1-40 days), although males who died had a mean stay of 4.3 days compared to females who died following a mean stay of 8.6 days. The average age of patients was 21.4 ± 1.9 years (n=247), and there was no significant difference between males (21.5 ± 2.5 years) and females (21.1 ± 3.7 years). The median age of male patients was 16 years, while for females the median age was 14 years. The youngest patient in the series was a child of 1 year, while the oldest was a man of 70 years.

The use of antivenom was recorded for just seven patients, and in all of these cases, CSL Polyvalent Australia/New Guinea Antivenom was administered. No data is currently available on the use of antivenom in patients who subsequently died. As patient files are still to be located for many of the cases under review, it is expected that further records of antivenom use will be located, and that this data combined with the clinical reports will substantially contribute to producing a comprehensive analysis of problems associated with the treatment of snakebite throughout Papua New Guinea.

Poster 1-34

BITES BY SPIDERS OF THE FAMILY THERAPHOSIDAE IN HUMANS AND CANINES

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Spiders of the family Theraphosidae occur throughout most tropical regions of the world. Bites by these spiders appear to cause only minor effects, but their hairs have been responsible for urticating skin reactions and ocular injuires. There have only been three single case reports of bites by these spiders previously[1] in Australia. The aim of this study was to describe the clinical effects of envenomation by Australian Theraphosid spiders in both humans and canines.

Cases were collected by the authors over the period January 1997 to October 2001, either prospectively in a large study of Australian spider-bites[2], or retrospectively from cases reported to the authors. Subjects were included if they had a definite bite and had collected the spider. The spider was identified by an expert arachnologist to species level where possible. There were 7 confirmed bites by spiders of the family Theraphosidae in humans and 3 in canines. This included bites by two *Selenocosmia* spp. and by two *Phlogiellus* spp. The 7 spider-bites in humans did not cause major effects. Pain and puncture marks were the commonest effects, with severe pain in 57% of cases. In one case the spider had bitten through a finger nail. Mild systemic effects occurred in one case. Clinical effects had resolved within 24 hours in all cases. There were 3 bites in dogs (*Phlogellius* spp. and *Selenocosmia* spp.), and in two of these the owner was bitten after the dog. In all cases the dog died, and in one case as rapidly as 2 hours after the bite.

This is the first series of bites by Australian Theraphosid spiders which, although not large, gives an indication of the spectrum of toxicity of these spiders in humans. Bites by these spiders are unlikely to cause major problems in humans. The study also demonstrates that the venom is far more toxic to canines and is a likely cause of some unexplained deaths in dogs in tropical parts of the world.

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Poster 1-35

SERUM SCORPION VENOM LEVEL AND CLINICAL VARIABLES ACCORDING TO SEVERITY GRADE IN PATIENTS TREATED WITH ALACRAMYN™

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Scorpion sting is a frequent accident in Mexico with over 180 000 cases reported yearly. The scorpion envenomation produces a pattern of neurotoxicity with a spectrum of severity ranging from trivial to life-threatening. Mild envenomation consist mainly of local pain and resolves without specific treatment. Severe envenomation, may involve neuromotor hyperactivity, pulmonary edema and ventilatory compromise, occasionally resulting in death. Alacramyn™ is a specific antivenom for *Centruroides sp* scorpion consisting of horse F(ab)2 fragments.

We conducted a clinical trial in 19 rural clinics. The objective was to describe the total hospitalization time, number of vials needed to reverse clinical syndrome and serum venom levels according to the grade of envenomation. Serum venom levels were measured with a specific ELISA assay. The product safety was also assessed.

105 patients entered the study, 36 females and 68 males; 29 of them were children and 76 adults. 42.6% had a mild, 44.9% a moderate and 12.5% a severe grade of intoxication. The hospitalisation time according to envenomation grade was 109 ± 57 minutes, 113 ± 57 minutes and 175 ± 102 minutes respectively. The average number of vials needed to reverse the clinical syndrome was 1.2 for mild, 1.7 for moderate and 2.9 for severe cases. At admission, the serum venom levels were 1126 ± 1152 pg/ml, 2014 ± 1209 pg/ml and 2105 ± 1685 pg/ml according to envenomation grade. The average percentage of serum venom reduction between admission and discharge is 95% for all envenomation grades.

Three patients presented transient adverse events, which were mild. None of these patients required longer than expected hospitalisation (hospitalisation time were 45, 61 and 65 minutes). No death occurred during the study period.

In conclusion Alacramyn™ effectively removed scorpion venom from the blood, and reversed the clinical envenomation syndrome.

Poster 1-36

PHARMACOKINETICS OF ANTISCORPION HORSE F(ab)$_2$ FRAGMENTS ALACRAMYN™ IN 6 HEALTHY VOLUNTEERS

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Scorpion sting envenomation is a frequent health problem in Mexico with over 180 000 cases reported yearly. The scorpion venom is a polypeptide that causes neurotoxicity enhancing nerve excitability. The clinical syndrome ranges from mild local symptomatology, to severe life-threatening alterations that might lead to death. Nevertheless, the mortality rate after envenomation has declined markedly during the past few years. This decline is attributable to a more systematic usage of antivenom in locations where intensive care units are not available. The rationale underlying the use of antivenom as the specific treatment for scorpion envenomation is based on its capability to bind and neutralise the venom. One important pharmacological parameter that influences the clinical effectiveness of the antivenom is its kinetic, which reflects how fast, how much and how long the venom-antivenom interaction takes place.

Alacramyn™ is a *Centruroides sp* specific antivenom composed of horse F(ab)$_2$ fragments. The objective of this study was to determine the pharmacokinetic and safety parameters of Alacramyn™ in healthy volunteers.

One vial of Alacramyn™ was administered as an IV bolus to 6 healthy male volunteers ranging from 20 to 26 years of age and 67 to 125 kg of weight. Blood F(ab)2 levels were measured with a specific ELISA assay. Blood samples were drawn at 5, 15, 30, 45, 60, 90, 120, 180, 360 minutes, at 24, 48, 72, 96 hours, and at 10 and 21 days after Alacramyn™ injection. A second blood sample was taken at baseline and 24 hours after Alacramyn™ infusion for clinical laboratory evaluation (complete blood count, chemistry, liver function test) and a questionnaire designed to evaluate the presence of serum sickness.

The distribution half live was 11.9 ±3.6 hr, elimination half live was 123.8 ± 38.9 hr, volume of distribution at steady state is 12120.7 ± 2615.2 ml and total clearance is 93.3 ± 32.4 ml/hr.

No laboratory abnormality nor serum sickness were found.

Poster 1-37

FINANCIAL IMPLICATIONS OF JELLYFISH ENVENOMING IN TROPICAL NORTH QUEENSLAND

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Jellyfish envenoming is an increasingly important cause of morbidity in Tropical North Queensland. The financial implications to the Emergency Services are also increasing. This presentation aims to identify the direct cost to the Emergency Services, namely Queensland Rescue, Queensland Ambulance Service, The Emergency Department and the in-hospital units of Cairns Base Hospital for the period from April 2001 to April 2002. The potential cost saving with effective preventative measures is highlighted.

Poster 1-38

THE INCIDENCE OF MYOCARDIAL INJURY IN HOSPITAL PATIENTS WITH IRUKANDJI SYNDROME

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Envenomation by the jellyfish *Carukia Barnesi* has the potential to produce the "Irukandji Syndrome". The symptoms include chest, back and abdominal pain, nausea and vomiting, sweating, muscular pains. Less commonly, myocardial injury can occur which may result in myocardial depression and pulmonary oedema. The incidence of myocardial injury in patients that attended Cairns Base Hospital Emergency Department with Irukandji syndrome is reported.

An audit of all patient presentations to the Cairns Base Hospital Emergency Department over one year with a discharge diagnosis of Irukandji syndrome. All patients with a discharge diagnosis of Irukandji syndrome from April 2001 to March 2002 were included in the study (n=126). Patients were assessed clinically and then haematological/biochemical analysis and Electrocardiograph (ECG), were performed if diagnosed with Irukandji syndrome. This included Full Blood Count (FBC), Electrolytes, Urea and Creatinine (EUCr), Cardiac Troponin I (cTnI) and Creatinine Kinase (CK).
Patients with a raised cTnI or CK, abnormal ECG or echocardiography results.
In the year April 2001 to March 2002 126 patients presented to Cairns Base Hospital Emergency Dept. and were diagnosed with Irukandji syndrome. 20% were found to have a raised cTnI and CK. Of patients with abnormal cardiac enzymes/ECG, 10% demonstrated abnormal myocardial function on echocardiogram.
20% of patients with Irukandji Syndrome were found to have evidence of cardiac insult.

Poster 1-39

A PROGNOSTIC SCORING SYSTEM FOR ACUTE MELIOIDOSIS

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Melioidosis, due to infection with the environmental organism *Burkholderia pseudomallei,* is still associated with a high mortality despite improvements in antibiotic therapy. Using simple clinical findings and baseline laboratory tests available at the time of admission, we wished to define a group with acute melioidosis at higher risk for death.

Using data collected prospectively over 12 years, a number of variables were selected as they were easily available at the time of admission and reflected organ dysfunction. The following variables were found to predict mortality in a univariate logistic model; the presence of pneumonia, age at diagnosis, serum urea and creatinine, serum bilirubin, lymphocyte count and serum bicarbonate. These variables were examined in more detail using a generalized additive model. A score was assigned from 0 to 2, based on the degree of abnormality. A melioidosis score was formed from the sum of these scores, with a maximum score of 11. A score of 4 or below (n=167) was associated with a mortality of less than 4%, whereas a score of 5 or above (n=95) was associated with a mortality of above 30%.

Although this scoring system requires validation, it may help identify a suitable target group for intensive intervention such as early ICU admission, the early use of meropenem and the adjuvant use of G-CSF. We intend to validate this scoring system prospectively and apply it in routine management as well as intervention studies.

Poster 1-40

TODAY'S TRAVELLER AND DIVING ADVENTURES - HOW TO SURVIVE YOUR DAY IN PRACTICE

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Travel broadens the mind, or so we are frequently told. The Australian public has certainly embraced the notion enthusiastically, and they are seen travelling more into remote and often unknown areas.

As travel doctor or the GP seeing travel patients, you will no doubt see many that wish to go for an adventurous trip and do some snorkelling and diving.
This presentation aims at highlighting some areas of medical check prior to the trip that must be considered in this group of travel patients.
Pitfalls in practice and warnings that must be given in order to prevent disasters from occurring in those of little experience, and what to advise in emergency cases 5,000 kilometres away from home.

Common accidents and problems while on holidays, and the follow up if any post-travel will be looked at. Areas such as diving specific problems, bites and stings, and the general health problems related to travel in the tropics will form the main group of potential worries to the unwary medical practitioner.

Poster 1-41

HEALTH ADVICE GIVEN BY GENERAL PRACTITIONERS FOR TRAVELLERS FROM AUSTRALIA

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Objectives: To investigate the prevalence of travel health advice and written documentation reported to be given by general practitioners to travellers from Australia.

Methods: A postal questionnaire was sent to 433 general practitioners GPs randomly selected from the register of the Medical Directory of Australia from the areas of western Sydney and Townsville.

Results: Two hundred and thirteen questionnaires (49.2%) were returned. Approximately two thirds of the sample was male (133/207, 64.3%) and one third female (74/207, 35.7%). The mean age of the GPs was 46.7 (SD±12.1) years. The GPs reportedly saw an average of 3.9 (SD±11.8) travellers per week. Most GPs (160/202, 79.2%) reported that they spent between 5-25 minutes for pre-travel consultations. GPs generally reported giving advice to travellers on travel vaccines, malaria prophylaxis, personal protective measures against insect bites, geographic diseases, clothing, and sexually transmitted infections. The majority of GPs did not routinely give information on travel insurance, unsafe sex, barotrauma, in-flight exercise, jet lag or first aid knowledge. Most GPs reported not routinely giving written documentation in the form of written travel health advice, a doctor's letter or a travellers' vaccination record.

Conclusions: GPs report seeking core information needed for formulating travel health advice. GPs also provided travellers with health advice on most of the areas, which need to be covered in the pre-travel consultation. More GPs may wish to consider advising travellers about the importance of travel insurance and managing common maladies of travellers, such as motion sickness, barotrauma, and jet lag. With limited time in general practice to advise travellers, more GPs should consider providing written advice and documentation for travel, including a travellers' vaccination record.

Poster 1-42

RESOURCES UTILIZED BY GENERAL PRACTITIONERS FOR ADVISING TRAVELERS FROM AUSTRALIA

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Background: General practitioners (GPs) use a number of resources to assist in the pre-travel consultation. This study investigated the prevalence of various resources utilized by GPs in advising travelers from Australia, including use of practice staff and referral, together with the prevalence of training of GPs in travel medicine.
Methods: In 2000, 433 GPs from western Sydney and Townsville were randomly selected from the register of the Australian Medical Association's *Medical Directory of Australia* database and sent self-administered questionnaires. Two reminders were sent.
Results: Two hundred and thirteen questionnaires (49.2%) were returned. Approximately two thirds of the sample was male (133/207, 64.3%) and one third female (74/207, 35.7%). The mean age of the GPs was 46.7 (SD±12.1) years. The national immunisation guidelines, contained in *Australian Immunisation Handbook*, and a commercial publication, *Travel Bugs*, were the most frequently used publications of GPs. About half of GPs reported having some formal training in travel medicine (47.1%, 98/208). About one tenth of GPs reported having a Yellow Fever Licence (11.3%, 23/203). The majority of GPs did not use their practice staff for giving travel health advice (60.7%, 122/201) or giving travel vaccinations (55.7%, 112/201). Almost half of GPs stated that they would refer to travel clinics at least sometimes (46.6%, 95/204), but most GPs would not refer to other agencies.
Conclusions: The most useful and probably the most accessible information resources used by GPs, possibly as they are provided free, were the *Australian Immunisation Handbook* and *Travel Bugs*, which between them outline many of the Australian recommendations for providing travel health advice. GPs refer travellers to a variety of sources for travel health advice, at least sometimes, especially to travel clinics.

158L-3

Poster 1-43

TRENDS IN ANTIMALARIAL DRUGS PRESCRIBED IN NEW ZEALAND 1993-1998

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***Introduction*:** Previous research has suggested considerable variability in the patterns of prescribing of antimalarial drugs. The aim of this study was to investigate the trends in use of antimalarial drugs prescribed in New Zealand (NZ) from 1993-1998.
***Method*:** In June 1999, IMS Health (NZ) Limited was approached to obtain retrospective data on antimalarials used in New Zealand for the period 1993-1998. This data was derived from the IMS Health (NZ) Limited New Zealand Pharmaceutical Index and New Zealand Hospital Index 1993-1998".
***Results*:** More than 200,000 patient weeks of antimalarials drugs were prescribed each year from 1993-1998. The most commonly prescribed antimalarials in New Zealand appeared to be quinine and mefloquine. These also accounted for the majority of the manufacturers' cost of antimalarials, excluding doxycycline, during the period. Maloprim® and related antifolate antimalarials had virtually ceased to be used in New Zealand by 1997. Proguanil gained some popularity once it became available in New Zealand, although it had a higher manufacturer's cost.
***Conclusions*:** This study has confirmed that travel health advisers have extensively prescribed antimalarials other than those recommended in official guidelines as malaria chemoprophylaxis during the period 1993-1998 in New Zealand. Quinine and mefloquine were popular choices amongst New Zealand travel health advisers. Withdrawal from the market of less effective antimalarials, such as Maloprim.®, appears to have been the most effective means of influencing the prescription of antimalarials, together with the issuing of updated National travel health guidelines. Continuing medical education, compliance with the national travel medicine guidelines and regulation of antimalarial use, especially through restricted access and withdrawal from the market, may be useful. Where appropriate, advice concerning personal protective measures against malaria and other vector-borne diseases should continue to be given.

158L-2

Poster 1-44

TRENDS IN ANTIMALARIAL DRUGS PRESCRIBED IN AUSTRALIA 1992-1998

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Background: Previous research has suggested considerable variability in the patterns of prescribing of antimalarial drugs. The aim of this study was to investigate the trends in prescription of antimalarial drugs prescribed in Australia from 1992-1998.
Methods: In 2001, data was extracted from the Australian Statistics in Medicine reports published by the Pharmaceutical Benefits Advisory Committee, Drug Utilization Committee, on prescriptions for antimalarials in Australia for the period 1992-1998.
Results: Over the 7-year period, the use of chloroquine and pyrimethamine/dapsone (Maloprim®) declined 81.0% and 98.9% respectively. Prescriptions for pyrimethamine alone declined by 52.0%. Prescriptions for mefloquine and proguanil increased 37% and 1,835% respectively. Prescriptions for quinine rose 10.6% while prescriptions for doxycycline fell 32.5%. Since quinine and doxycycline are widely used for purposes other than prevention and treatment of malaria, it was difficult to comment on their possible use as antimalarials.
Conclusions: This study has shown a marked fall in pyrimethamine combination antimalarial drugs over the years 1992-1998 with a rise in mefloquine and proguanil. The prescribing in 1998 of 967 courses of Maloprim is of concern since there is now no justification for use of this combination as a chemoprophylactic agent. The issuing of updated National travel health guidelines appears to have been the most effective means of influencing the prescription of antimalarials, with considerable reduction in the use of antifolate drugs. Continuing medical education, compliance with the national travel medicine guidelines and regulation of antimalarial use, especially through restricted access and withdrawal from the market, may be useful in maintaining appropriate prescription of antimalarial drugs. Where appropriate, advice concerning personal protective measures against malaria and other vector-borne diseases should continue to be given.

158L-4

Poster 1-45

THE PREVALENCE OF LOW BACK PAIN AND RELATED DISABILITY IN AUSTRALIAN ADULTS

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Estimates of low back pain prevalence show that low back pain is a common problem particularly in western countries. But the extent to which low back pain causes true disability and not just nuisance pain castes doubt of the utility of these estimates. The objectives of this study were to determine the prevalence ranges of low back pain together with any related disability in Australian adults. A survey was mailed to a stratified random sample of 3000 Australian adults selected from the Electoral Roll. There was a 69% response rate. Demographic variables of respondents were compared with those of the Australian population taken from Census data. Selective response bias was investigated using wave analysis. A range of prevalence data were derived as was a disability score using the Chronic Pain Grade Questionnaire[1] (CPG). The CPG has demonstrated reliability and validity in measuring pain and disability in postal surveys[2]. Prevalence and disability estimates were variously standardised using gender, age and marital status.

There was little variation between the sample and the Australian adult population. There was no significant selective response bias found. The sample point prevalence was estimated at 25.5% (95% CI, 23.6-27.5), six-month prevalence was 64.6% (95% CI, 62.6-66.8) and lifetime prevalence was 79.2%, (95% CI, 77.3-80.9). The "retrospective 1-year incidence" was 8.0% (95% CI, 6.9-9.3). In the previous 6-month period 42.6% (95% CI, 40.4-44.8) of the adult population had experienced low intensity pain and low disability from it. Another 10.9% (95% CI, 9.6-12.3) had experienced high intensity pain, but still low disability from this pain. However, 10.5% (95% CI, 9.2-11.9) had experienced high disability low back pain. The mean time-off from usual activities in the past 6-months for this group was 1.6 months (95% CI, 1.3-1.9), the median was 18 days. There was no gender difference for a high disability rating or time-off. Low back pain is a very common problem in the Australian adult population, yet most of this is low intensity and low disability pain. Nevertheless, over 10% had been disabled by low back pain in the past 6-months and it required significant time off from usual activities.

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Poster 2-1

SOME TOXIC AND IMMUNOLOGIC STUDIES ON *LOXOSCELES* VENOM GLAND HOMOGENATES FROM SPIDERS OF SOUTH AMERICA AND NORTH AMERICA.

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Bites by *Loxosceles* spiders may produce envenoming with important loss of tissue and systemic disorders that can lead to death. As the beginning of a study on biochemical characteristics of venom from *Loxosceles* (*L.*) spiders from South and North America and on the neutralizing capacity of different antivenoms (AV), some biochemical and toxic activities of venomous gland homogenates of *L.laeta* from Argentina and *L.boneti* and *L.reclusa* from México and U.S.A. were studied. SDS-PAGE was performed and the necrotizing and lethal potencies were studied. Additionally the immunochemical reactivity and the neutralization of toxic activities between venoms and AV were studied. The SDS-PAGE showed strong stained bands in the order of the 60-65, 30-35 and 20-25kDa, and there were observed differences in the intensity of staining in some bands when both venoms were compared. The minimal necrotizing dose (dose of venom that produces a necrotic-hemorrhagic area of 1.0 cm^2) in New Zealand rabbits was around 1.5 ± 1.0μg for *L.boneti* venom and around 3.0 ± 1.0μg for *L.laeta* venom. The gross pathological examination showed differences in the type of lesions, displaying good delimitated necrotic-hemorrhagic lesions with a central area of necrosis 48 hs following the *L.boneti* venom injection, while *L.laeta* venom caused gross hemorrhagic necrotic lesion, high compromise of the subcutaneous tissue with important edema and central necrosis of slightly minor intensity. The lethal potency in mice (CF-1, 20g) was 19μg (c.i.17-22μg) for *L.boneti* venom and 40μg (c.i.20-60μg) for *L.laeta* venoms. The ELISA and Ouchterlony studies showed high cross reactivity using homologous or heterologous AV displaying the first slightly higher reactivity. Anti-*L.gaucho* antivenom fully neutralized the necrosis produced by 10 MND of *L.boneti* venom at a dose of 20μl and the lethality by 3 LD_{50} at a dose of 100μl but it was less effective in neturalizing the lethality and necrosis of *L.laeta* venom than the homologous anti *L.laeta* AV. Anti-*L.laeta* AV showed different neutralizing capacity depending the AV tested. This preliminary results suggest that heterologous anti-*Loxosceles* AV could neutralize the toxic activities of *Loxosceles* venoms not used as immunogen even from spiders of different latitude, as has been observed with venoms from South American (1) and North American (2) *Loxosceles* spiders.

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Poster 2-2

CHEMICAL CHARACTERIZATION OF THE MAJOR COMPONENTS OF THE CRUDE VENOM FROM THE SPIDER *Macrothele gigas* (Hexathelidae)

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The major neurotoxins from the crude venom of the spider *Macrothele gigas* from Iriomote, Japan, were chemically characterised. The venom of the Hexathelidae spider was purified by reversed-phase and cation exchange HPLC. The crude venom contained a high concentration of a polyamine and six major neuropeptides. The chemical structure of the polyamine (Mg30) shows some similar identity to those polyamines from the tarantula spider *Harpactirella sp.* (Theraphosidae). The six major peptides from *M. gigas* were alkylated and partially sequenced. Three peptides are reticulated by 3 disulfide bridges (DB), two are folded by 4 DB and one is plaited by 5 DB. The peptides with 3 DB share some identity to those potassium channel blockers from the spiders *Heteropoda venatoria* (Sparassidae) and *Phrixotrichus auratus* (Theraphosidae). One of the peptides with 4 DB shares some identity those peptides atracotoxin and versutoxin which block both insect and mammal sodium channel from the Australian spiders *Atrax robustus* and *Atrax versuta* (Hexathelidae) respectively. The peptide with 5 DB share some identity to those peptides Tx2 and Tx4 from the Brazilian spider *Phoneutria nigriventer* (Ctenidae).

Poster 2-3

Structure Characterization of Spider Toxin Stored in the Venom of *Nephila madagascariencis* and *Macrothele gigas*

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Spider venoms contain many compounds like proteins, peptides, amines, amino acids and acylpolyamines that are nonproteinaceous class of neuroactive compounds. The acylpolyamines can be devided into two groups, the amino acid-containing acylpolyamines and the non-amino acid-containing acylpolyamines, both types function similarly as glutamate receptor blockers, and also having the same family of aromatic end groups.

We have confirmed the existence of twenty-two amino acid-containing acylpolyamines from the spider venom of *nephila madagascariencis* using matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) and high-energy collision induced dissociation (CID) four-sector tandem mass spectrometry. Among those acylpolyamines, seven compounds were not known previously. The structures of six compounds were determined by mass spectrometric approach. The compounds NPTX-466B and NPTX-643F that have the same molecular weights and formulas with JSTX-1 and NPTX-9, respectively, were found out to be the isomeric form of structures. In addition to the previously reported generalized polyamine backbone structures from type-A and –E, new acylpolyamines that have type-F backbone were characterized.

On the other hands, the non-amino acid-containing acylpolyamines named MG-30 was isolated from the venom of *Macrothele gigas* collected at Iriomote-island in Japan.

Poster 2-4

THE IDENTIFICATION, CHARACTERISATION AND STRUCTURE DETERMINATION OF A GROUP OF MU-AGATOXIN-LIKE COMPONENTS FROM THE VENOM OF AUSTRALIAN FUNNEL-WEB SPIDER SPECIES.

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The increasing study of spider venoms has shown they contain a complex array of toxins, and thus provide a rich natural resource of pharmacologically active components. The specific activities these toxins display can be exploited in the development of selective biochemical or pharmacological tools, and/or provide leads in the discovery of therapeutics and insecticides.

The Australian Funnel-web spiders are a group of venomous arachnids isolated to the south east coast of Australia and currently consist of 35 characterised species belonging to two genera (*Atrax* and *Hadronyche*) (1). Envenomation by species of this group of spiders has resulted in thirteen recorded human fatalities earning the Funnel-web spider a formidable reputation worldwide (2).

Here we describe the protocol for the rapid identification and characterisation of a group of toxins from species of the Australian Funnel-web spiders exhibiting similarities to the mu-agatoxins from the American Funnel-web spider through a combination of RP-HPLC/UV, RP-HPLC/ESI-MS and molecular biology RACE techniques. This group of hydrophobic toxins was found to consist of two toxin families differing considerably in amino acid composition and also length. Furthermore, the structure determination of a member from one family illustrated a structural similarity to the mu-agatoxins, in particular to mu-agatoxin IV which is present in two conformations resulting from the *cis-trans* isomerisation of Pro15 (3).

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Poster 2-5

STRUCTURE-FUNCTION STUDIES OF THE INSECT-SPECIFIC CALCIUM CHANNEL BLOCKER ω-ACTX-Hv2a

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Insect pests decimate a significant proportion of the world's food supply and transmit a number of debilitating and deadly human diseases [1]. These arthropods are generally controlled by spraying broad-spectrum chemical insecticides. However, the emergence of insecticide-resistant insect populations and increasing concern about the environmental and human health risks associated with certain agrochemicals has stimulated the search for new arthropod-control strategies.

Since the primary role of spider venoms is to kill or immobilize arthropod prey, it is not surprising that spider venoms have proved to be rich sources of insecticidal compounds. We have discovered several insecticidal neurotoxins by screening the venom of the Australian funnel-web spider *Hadronyche versuta* [2], including a potent blocker of voltage-gated calcium channels (VGCCs) that we named ω-ACTX-Hv2a [3]. This toxin appears to have >10,00-fold specificity for invertebrate versus vertebrate VGCCs, making it an attractive lead compound for insecticide development [3].

Structural characterization of the toxin revealed a 45-residue peptide with a highly compact globular domain (residues 1-32) containing a classical inhibitory cystine knot motif. In marked contrast to this highly ordered disulfide-rich core, the entire lipophilic C-terminal "tail" (residues 33-45) is disordered in solution. Deletion of this apolar tail completely abrogates toxin function [3]. In order to probe the role of individual residues in toxin function, and to elucidate the function of the structurally disordered tail, we have examined the activity of a number of natural and synthetic C-terminal deletions of the toxin. We are also developing an *Escherichia coli* expression system for production of recombinant ω-ACTX-Hv2a so that the structure-function relationships can be probed in more detail using site-directed mutagenesis. We will also present results from both biochemical and genetic screens designed to elucidate the exact molecular target of the toxin.

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Poster 2-6

FEMALE WOLF SPIDER (*LYCOSA* spp.) CRUDE VENOM MODULATES [^{3}H]-DOPAMINE RELEASE IN THE RAT CAUDATE PUTAMEN AND MAY CONTAIN A MYOTOXIC COMPONENT

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The wolf spider (*Lycosa* spp.) is a large, sometimes aggressive, nocturnal hunter found throughout the temperate regions of Australia. Common symptoms of envenomation by Australian wolf spiders include local pain, swelling and erythema. They have also been implicated in the controversial syndrome, necrotic arachnidism, which may be due to the presence of a myotoxic component(s) in their venom.

In this study, female wolf spider crude venom (100μg/ml) produced an increase in baseline and a reduction in the twitch height of the isolated, nerve-stimulated chick biventer cervicis nerve-muscle preparation (n=4). This effect was time dependent and believed to be indicative of myotoxicity. Further, preliminary histological studies also support the existence of a myotoxic component in this venom.

The effect of female wolf spider crude venom (50μg/ml) on [^{3}H]-dopamine (DA) release from isolated prisms of rat caudate putamen (CPu) using *in vitro* superfusion was also investigated. We show for the first time female wolf spider venom (50μg/ml) produces a significant increase in basal [^{3}H]- DA from prisms of rat CPu (+108%; $p<0.05$). Interestingly, this effect was not dependent on the presence of extracellular Ca^{2+}. In the presence of extracellular Ca^{2+}, no difference in basal [^{3}H]- DA release was seen in response to the addition of crude venom. The K^+-stimulated release of [^{3}H]- DA from prisms of rat CPu was facilitated by the addition of female wolf spider venom (50μg/ml) in the absence of extracellular Ca^{2+} (+164%, $p<0.001$). However this effect was not seen with the crude venom in the presence of Ca^{2+}. These findings suggest that the modulation of basal and K^+-stimulated release of [^{3}H]- DA from the rat caudate putamen by female wolf spider crude venom is largely Ca^{2+}-independent. Whether or not the toxin(s) responsible for these effects is the same as that implicated in the skeletal muscle studies is yet to be elucidated.

Poster 2-7

MODULATION OF SODIUM CHANNEL GATING AND KINETICS BY δ-MISSULENATOXIN-Mb1a FROM THE AUSTRALIAN EASTERN MOUSE SPIDER *MISSULENA BRADLEYI*

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The male Australian eastern mouse spider (*Missulena bradleyi*) remains a potential cause for serious envenomation in humans. Symptoms of envenomation are similar to those reported for Australian funnel-web spider envenomation and include tachycardia, dyspnea and profuse sweating. To identify the neurotoxin responsible for envenomation, whole male venom was fractionated using analytical C_{18} reverse-phase HPLC. Neurotoxic fractions were identified using an isolated chick biventer cervicis nerve-muscle preparation as a bioassay. The major neurotoxic fraction caused an increase in resting tension, muscle fasciculations and a decrease in indirectly stimulated twitch contractions. The responses of exogenously applied acetylcholine or KCl were unaffected in the presence of the toxin. Moreover the toxin-induced contracture was unaltered by prior incubation with (+)-tubocurarine, however all responses could be inhibited by tetrodotoxin (TTX). This indicates that the toxin targets presynaptic nerve structures resulting in spontaneous neurotransmitter release. Interestingly, these effects were neutralised by antivenom raised against the venom of the Sydney funnel-web spider *Atrax robustus*. These actions are similar to those seen with crude venom (1).

Whole-cell patch clamping electrophysiology of dorsal root ganglion (DRG) revealed that the toxin caused a slowing of TTX-sensitive sodium channel inactivation, and a hyperpolarizing shift in the threshold of activation, in a manner similar to δ-atracotoxins isolated from Australian funnel-web spiders. Accordingly the toxin was named δ-missulenatoxin-Mb1a (δ-MSTX-Mb1a) using the nomenclature previously described for Australian funnel-web spider toxins with 'δ' prefix reflecting a major action to slow sodium current inactivation.

Electrospray ionisation mass spectrometry gave a molecular weight of 4959 ± 0.3 Da for the native molecule and 5808 ± 0.35 Da for the pyridylethylated toxin, the increase in mass corresponding to the derivitisation of eight cysteine residues. No free cysteine residues were detected indicating that the native toxin has four disulphide bonds. A partial N-terminal sequence (35 residues) determined by Edman degradation and MS/MS revealed that, despite expectations, δ-MSTX-Mb1a shows only 20% homology with δ-atracotoxins and low homology with other site-3 neurotoxins, despite these toxins having similar activity on voltage-gated sodium channels.

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Poster 2-8

CHARACTERIZATION OF THE VENOM COMPONENTS IN SPIDER WASPS

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Solitary wasps paralyze other insects or spiders by using their venom for feeding their larvae. Therefore, the venom of solitary wasps are believed to contain a variety of neurotoxins. In our previous study, several novel peptides were identified from the venom of solitary wasps. Pompilidotoxins (PMTXs), which inhibit the Na+ channel inactivation, were obtained of the venom from spider wasps *Anoplius samariensis* and *Batozonellus maculifrons* (family: Pompilidae). On the other hand, wasp kinin-like peptides were found in the venom of *Cyphononyx dorsalis*. Additionally, we have developed the mass spectrometric method for the determination of the minor peptides in the wasp venom (1, 2). The structural determination of these peptides from spider wasps will be discussed.

However, the wasp venom consists of a complex mixture of not only peptides, but also proteins and low molecular weight compounds. In this study, we also characterized the low molecular weight compounds of the venom of *A. samariensis* and *B. maculifrons* by semimicro-LC/MS (3) and NMR analysis. The venom of both wasps mainly contained GABA, glutamic acid and alanine. In addition, several biogenic amines and nucleoside derivatives were identified by LC/MS analysis. The venom of other Pompilidae wasps and *Chalybion japonicum,* which hunt spiders but dose not belong to Pompilidae family, were also analyzed and compared.

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Poster 2-9

APPLICATION OF MAIDL-TOF MS AND LC-MS/ LC-MSMS IN CHARACTERIZING NOVEL PEPTIDES IN SCORPION VENOM

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Scorpion venom is a cocktail mixture containing various biological active compounds. The medical interest components mostly include peptides and polypeptides with molecular weight between 700 Da and 10 kDa. In one-time stimulation of crude venom from an individual scorpion, more than 70 different peptides may exist in only around 2 μl collection. Application of MALDI-TOF MS and LC-MS/LC-MSMS in screening novel peptides from the complex profile and characterizing their structure is described in this report. It requires less amount sample and makes characterization interest peptides from one individual scorpion venom into possibility.

With this method, we successfully identified the structure of several novel short peptides from two species of scorpions: *Opisthacanthus madagascariensis* from Madagascar and *Isometrus evropaevs* from Japan. One group are linear amphipathic antimicrobial peptides. The second group are linear peptides rich in hydrophobic amino acid residues that may effect cell signalling system. The third group are unusual acidic peptides with two disulfide bridges.

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Poster 2-10

TOXINS AND GENES FROM THE VENOM OF THE ASIAN SCORPION *BUTHUS MARTENSI* KARSCH

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Among the different scorpion species, *Buthus martensi* Karsch (*Bm*K), a widely distributed scorpion species in Asia, has received a lot of attention. Indeed, over the past decade, more than 70 different peptides, toxins or homologues have been isolated and more peptides are probably still to be revealed. We have focussed on the many peptides isolated from the venom of this scorpion, their targets, their genes and their structures. The aim is to give both a 'state of the art' view of the research on *Bm*K venom and an illustration of the complexity of this scorpion venom. In the present communication, we have listed the different ion channel toxins and homologues isolated from the venom of *Bm*K, either from the literature or from databases. We describe here 51 long-chain peptides related to the Na^+ channel toxins family, 34 related to the α-toxin family, 4 related to the excitatory insect toxin family, 10 related to the depressant insect toxin, one β-like toxin plus 2 peptides, *Bm*K AS and AS1, that act on ryanodine receptors. We also list 18 peptides related to the K^+ channel toxin family: 14 short chain toxins or homologues, 2 long chain K^+ toxin homologues and 2 putative K^+ toxin precursors. Additionally, two chlorotoxin-like peptides (*Bm*-12 and 12b) have been isolated in the venom of *Bm*K. Besides these ion channels toxins, 2 peptides without disulfide bridges (the bradykinin-potentiating peptide *Bm*K bpp and *Bm*K n1) and 3 peptides with no known functions have also been discovered in this venom.

We have also taken the opportunity of this study to update the classification of scorpion K^+ toxins (1), which now presents 17 subfamilies instead of the 12 previously described. The work on the venom of *Bm*K led to the discovery of 2 new subfamilies, α-KTx14 and α-KTx17.

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Poster 2-11

DYNAMIC DETERMINATION AND POSSIBLE MECHANISM OF AMINO ACID TRANSMITTER RELEASE FROM RAT SPINAL DORSAL HORN INDUCED BY THE SCORPION VENOM AND A NEUROTOXIN (BMK I)

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In the present communication, we determined the dynamic release of amino acid transmitters from spinal dorsal horn induced by scorpion BmK venom and a neurotoxin (BmK I). The results found that glutamate and aspartate release could be evoked significantly within the initial 30 minutes with the applied doses of either 0.05 and 0.01mg BmK venom or 0.01 and 0.002 mg BmK I. However, GABA release could be laggardly evoked during the second 30 minutes by the venom, but not by BmK I. The result suggested that nociceptive afferent fibers could be activated to induce excitatory amino acid release from spinal dorsal horn by nociceptive factors such as BmK I, but the delaying release of GABA might be attributed to modulating role of some antinociceptive components in the venom.

Poster 2-12

THE INHIBITORY EFFECTS OF BmK IT_2 ON NOCICEPTIVE BEHAVIOR AND C-FOS EXPRESSION IN RAT SPINAL DORSAL HORN INDUCED BY FORMALIN AND A POSSIBLE MECHANISM

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The present study, the inhibitory effects of BmK IT_2, a neurotoxin purified from scorpion *Buthus martensi* Karsch (BmK) venom on rat nociceptive response induced by 2.5% formalin have been investigated. Two-phase tonic nociceptive response and the expression of c-Fos in spinal dorsal horn could be induced by plantar injecion of fomalin into the rat hind paw. The spontaneous nociceptive behaviors was decreased significantly ($p<0.05$) in the second phase by co-adiministrated doses of 0.1 and 1μg BmK IT_2 with formalin. In addition, the expression of c-Fos in L4-5 segment of spinal dorsal horn was markedly inhibited at 2 h ($p<0.05$). It also was found that BmK IT_2 could specifically bind to axolemma-enriched fraction of rat sciatic nerves. The results suggested that the antinociceptive effects of BmK IT_2 may be partially attributed to the modulation of voltage-gated Na^+ channels in peripheral nerve terminals.

Poster 2-13

c-FOS EXPRESSION IN RAT SPINAL CORD INDUCED BY SCORPION BMK VENOM VIA PLANTAR SUBCUTANEOUS INJECTION

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The aim of this study was to assess the c-Fos expression in rat spinal cord induced by scorpion BmK venom. BmK venom was plantarly injected into one hind paw of the awake rats. Fos-like immunoreactive neurons were found to distinctly distribute at L4-5 segments after BmK venom application. The dense of c-Fos labeling was most in medial half portion of laminae I-II, and considerable in laminae V-VI, but a few in laminae III-IV, VII-X. c-Fos labeling could be detected at 0.5 h, reached the peak at 2 h, decreased steeply from 4 h, and then almost disappeared at 24 h. 10-50. □g BmK venom was deemed to be valid dosage to evoke c-Fos expression. Meanwhile, c-Fos expression induced by BmK venom could be suppressed partially by system morphine in a dose dependent manner. Peripheral administration of BmK venom can result in prolonged tonic pain-related responses in central neurons. The different extent activities of neuronal subpopulation in spinal cord involved in nociceptive transmission manifesting as c-Fos expression, were mainly correlated with the mechanisms underlying the generation, maintenance, and/or modulation of spontaneous pain and hyperalgesia evoked by BmK venom.

Poster 2-14

BIOSENSOR BINDING OF BMK ABT, A UNIQUE NEUROTOXIC POLYPEPTIDE ON MAMMAL BRAIN AND INSECT SODIUM CHANNELS

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The binding properties of BmK abT, a unique neurotoxin from Chinese scorpion, on sodium channels of mammal and insect neuronal cell membranes have been investigated using BIAcore assay in the study. Results showed that BmK abT could bind to both rat brain synaptosomes (the association rate constant about 2.49×10^{6} $M^{-1}s^{-1}$ and the dissociation rate constant about $1.57\times10^{-4}s^{-1}$) and Heliothis nerve cords synaptosomes (the association rate constant about 1.21×10^{7} $M^{-1}s^{-1}$ and the dissociation rate constant about $0.99\times10^{-3}s^{-1}$). The binding of BmK abT on rat brain synaptosomes could be partially inhibited by increasing membrane potential, but not by BmK AS, BmK IT2 and BmK I or modulated by veratridine. In addition, the binding of BmK abT on Heliothis nerve cords synaptosomes could be significantly enhanced by increasing membrane potential and veratridine concentration, inhibited by BmK IT2, but not by BmK AS or BmK I. The results suggest that BmK abT can bind to a distinct receptor site on mammal brain sodium channels, and associate with a related site of depressant insect-selective toxins on insect sodium channels.

Poster 2-15

CLONING OF cDNAS ENCODING SHORT INSECTOTOXINS FROM *MESOBUTHUS TAMULUS.*

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Mesobuthus tamulus is one of the most lethal scorpion species on the Indian sub-continent. Its envenomation leads to fluctuating blood pressure, pulmonary oedema, cardiovascular failure and possible death. Several sodium and potassium channel toxins have been isolated and characterized from the venom of this species. In comparison, very few natural toxins have been identified which target chloride ion channels. The most extensively studied chloride channel toxin is Chlorotoxin from the scorpion, *Leiurus quinquestriatus*. To date, less than twenty related proteins have been identified and are classified as short insectotoxins, although for the majority the toxicity and site of action remain to be elucidated.

PCR on an uncloned cDNA library of *M. tamulus,* using a gene specific forward primer corresponding to the 5' UTR of *Buthus martensi Karsch* insectotoxin Bm-12, and a reverse adaptor primer (Clonetech, USA), yielded several cDNA sequences. Two novel clones, BTChl1 and BTChl2 identified have been found to encode probable short insectotoxins having 36 and 38 amino acids respectively. BTChl1 and BTChl2 show 70% and 75% homology to Chlorotoxin and form the first insectotoxin cDNAs to be isolated from *M. tamulus*.

Poster 2-16

TAMAPIN: A PEPTIDE FROM THE VENOM OF THE INDIAN RED SCORPION (*MESOBUTHUS TAMULUS*) WHICH TARGETS SK CHANNELS AND AHP CURRENTS IN CENTRAL NEURONES

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Scorpion toxins are invaluable tools for investigating the structure and function of ion channels. The Indian red scorpion, *Mesobuthus tamulus* is the medically most important species of scorpion on the Indian sub-continent and in comparison with our knowledge of North African and American scorpions, our knowledge of the pharmacologically active peptides present in Indian scorpion venoms is very limited.

Tamapin is a new apamin-like peptide that we have recently identified in *Mesobuthus tamulus* venom. Tamapin (M_r 3458) inhibits ^{125}I-apamin binding to rat synaptosomal plasma membranes (K_I = 10pM). The toxin has 6 cysteine residues and its amino acid sequence is homologous to scyllatoxin and other scorpion venom toxins that block apamin-sensitive, low conductance Ca^{2+}-activated K^+ channels (SK channels).

When applied to CA1 pyramidal neurons on brain slices, tamapin (10 nM) selectively blocked the apamin-sensitive, medium duration afterhyperpolarizing current (I_{AHP}; 98.2±1.2% block; n=5), but had no effect on the apamin-insensitive slow AHP current. In current clamp, tamapin reduced the medium AHP and caused an attenuation of spike frequency adaptation in CA1 neurons. To test the effect of tamapin on cloned SK channels with defined subunit compositions, SK1,SK2 and SK3 channels were stably expressed in HEK293 cells and the effects of tamapin studied using the patch clamp technique in the whole cell configuration. Tamapin blocked all three currents, although the affinity of the toxin for the individual channels varied over three orders of magnitude (SK1, IC_{50} = 42nM; SK2, IC_{50} = 25 pM; SK3, IC_{50} = 1.7nM). Tamapin is 1.5-fold more potent than apamin and 10-fold more potent than scyllatoxin in blocking SK2 channels.

This work was supported by grants from the Wellcome Trust, the National University of Singapore, SFB406 and the Human Frontier Science Program.

Poster 2-17

Expression and purification of *Clostridium botulinum* type C and D neurotoxin heavy chain subunits

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Botulism is an intoxication caused by neurotoxins produced by *Clostridium botulinum* bacteria. The heavy chain of the clostridial neurotoxins, specifically the 50 kilodalton carboxy-terminal end of the proteins, have been shown to play a predominant role in toxin binding and could form the basis of a subunit vaccine. Previous studies, on tetanus (1, 2) and botulinum type A neurotoxins (3) have investigated the expression of these regions in *E. coli*. Due to the extreme A+T richness of the clostridial genome and the resultant codon bias, expression of these fragments in *E. coli* has generally been problematic.

In this study various subunits of the BoNT/C and D were expressed in *E. coli,* with a 6-histidine amino-terminal tag. Expression could not be detected on Coomassie stained SDS polyacrylamide gels but could be observed by Western blot analysis. Expression at 37°C resulted in low-level expression with significant amounts of lower molecular mass proteins also being present. Optimisation of expression conditions, specifically lowering of expression temperature to 25°C, resulted in a significant reduction in the level of the smaller proteins. Expression in one litre cultures resulted in yields of approximately 0.5 mg purified protein, with purification being carried out under denaturing conditions and proteins eluted at pH 5.9. Decreasing the pH of elution to 4.5 resulted in significantly higher yields of purified protein, in the range of 2.0-2.5 mg/l of culture. However, under these conditions the proteins formed a precipitate during dialysis against PBS. Precipitated proteins were recognised by anti-BoNT serum and were stable for up to one month at 37°C. These purified recombinant proteins were further investigated for their usefulness as vaccine antigens.

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Poster 2-18

THE *IN VITRO* ANTI-SNAKE VENOM STUDIES OF POLYPHENOLS FROM THAI MEDICINAL PLANTS

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The presence of polyphenols (tannins) in plant materials may inhibit energy activity by precipitation of enzyme proteins. The degree of inhibition is proportional to the polyphenol concentrations. In this study, tannin contents and types of four selected Thai medicinal plants were evaluated. Their effectiveness against the activities of *Naja kouthia* venom were quantitatively determined by *in vitro* methods. Precipitation of the venom proteins was induced by mixing the venom and various concentrations of the plant extracts and incubated the mixture at 37°C for 1 h. The supernatant and the precipitate were separated and characterized for the presence or absence of the venom proteins by SDS-PAGE method. Anti-lethal activity was determined in Swiss albino mice by injected the supernatant part through the tail vein and observing the percentage of their survival after 24 h. The effectiveness against acetylcholinesterase activity was determined by the enzyme assay.

It was found that the extracts of *Pentace burmanica* Kurz (PB), *Pithecellobium dulce* Benth. (PD) and *Areca catechu* Linn. (AC) contained both hydrolysable and condensed tannins while the extract of *Quercus infectoria* Olive. (QI) contained only hydrolysable tannins. Their total tannin content was between 0.44-34%. The extracts, which contained condensed tannins, could inhibit acetylcholinesterase activity and lethal activity at much lower tannin concentrations than the extract, which contained solely hydrolysable tannins. The effective dose (ED_{50}) of tannin against lethal activity ($4LD_{50}$) varied depending on the content of condensed tannin in the extracts. It was between 63-185 mcg per mouse for AC and PB extracts but for QI extract, was almost five to ten times higher. This could be explained due to the complex formation between condensed tannins and the venom proteins. This might consume less energy and therefore precipitate easier than hydrolysable tannins. Structural models of the complexation between neurotoxin and condensed tannins or hydrolysable tannins with minimum energy consumption were proposed by the assisted of Chem 3D, version 3.5.1 MM2 force field. The obtained results demonstrated the possibility of using these medicinal plants for topical first aid treatment of snakebite.

Poster 2-19

ANTI-NECROTIZING ACTIVITY OF TANNIC ACID AND THAI MEDICINAL PLANTS CONTAINING TANNIN AGAINST THAI COBRA VENOM

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Cobra bite is one of the major medical problems in Thailand. In addition to paralysis, local severe pain is an almost constant feature of an effective cobra bite. If the venom can be rapidly detoxified, local necrosis in the cobra bite may be inhibited. Tannin was reported to be a naturally occurring plant product which exerts inhibition on myonecrosis, haemorrhage and edema activities in mice. This is a preliminary study of the anti-necrotizing activity of tannic acid and Thai medicinal plants containing tannin. The anti-necrotizing activity was examined by mixing Thai cobra venom and various concentrations of tannin. The mixture was incubated at 37 °c, 1 h and then centrifuged. One hundred microliters of each supernatant containing one minimum necrotizing dose (MND) of venom was injected intradermally into rats. After 72 hours of injection, the lesion on the inner surface of rat skin was measured. The amount of tannin, which caused the necrotic lesion to disappear, was recorded. When 30 μg of tannic acid was used, the lesion disappeared. Anti-necrotizing activities of Thai medicinal plants; *Quercus infectoria, Pithecellobium dulce, Pentace burmanica* and *Areca catechu* , containing tannin ,were observed. *Quercus infectoria, Pithecellobium dulce* and *Areca catechu* showed the same neutralizing capacity against necrotizing activity of Thai cobra venom whereas a less neutralizing capacity was determined in *Pentace burmanica*. The results from this study may be useful in developing anti-snake venom preparations against local necrosis in cobra bite.

Poster 2-20

INFLUENCE OF CLINOPTILOLITE ON THE TOXIC EFFECTS OF MYCOTOXIN AUROFUSARIN IN JAPANESE QUAILS.

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The fungus *Fusarium* produces a range of mycotoxins (T-2 toxin, DON, zearalenon etc). However, there is a group of pigments which are also produced by *Fusarium* species which have, until recently, been ignored. Aurofusarin – is a dimmeric naphtoquinone meatbolie produced by Fusarium graminearum. Studies by Kotyk (1999) at the Ukrainian Poultry Research Institute over the last 10 years with the pigment, aurofusarin, demonstrate that this compound should be included in the list of important *Fusarium* mycotoxins. The results of our previous research have clearly shown the changes of quails eggs qualty as a results of aurofusarin consumption (decreased DHA proportion in egg yolk lipids and increased linoleic acid concentration, concentrations of vitamin E, carotenoids and vitamin A in the egg yolk also decreased; fertility and hatchability of aurofusarin enriched eggs was decreased). An experiment was conducted to examine influence of clinoptilolite (CLI, a natural zeolite) on the the impact of *Fusarium graminearum* dimeric naphthoquinone aurofusarin (AU) in Japanese quails. Four groups (control, AU, CLI, AU plus CLI) were formed from 45-day-old Japanese qualis. The experimental group qualis were fed a diet containing *Fusarium graminearum* culture (3%) containing exclusively aurofusarin at a level of 880 mg/kg, with and without 3% CLI. The experiment lasted 4 weeks. The performance of quails and eggs quality were measured. It was evaluated the aurofusarin treatment didn't alter egg productivity, but the fertility and hatchability of eggs were significantly decreased. The quality of eggs was reduced – the syndrome of "reducing egg quality" (color of egg yolk was brown with bloody spots) was observed. The content of vitamins E (α-tocopherol γ-tocopherol, α-tocotrienol and γ -tocotrienol), content of retinol and carotinoids were reduced. The addition of CLI to an AU-containing diet significantly reduced the effect of AU on the organism of Japanese quails and the egg quality. CLI treatment (3%) improved the hatchability by 13%, fertility - by 10%. The syndrome of "reducing of eggs quality" was not as severe as in the experimental group without CLI – the changing of yolk color was not so severe. The content of vitamins E was increased by 10,2%, retinol – by 13,14%, carotenoids – by 9,4%.

Poster 2-21

GENE EXPRESSION CHANGES OF CULTURED HUMAN LIVER CELLS EXPOSED TO AFLATOXIN B1

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Aflatoxin is a hepatotoxin produced by some strains of fungus (Apergillus), which develop in peanuts seeds. Aflatoxin B1 was prevalent which has carcinogenic effect on liver. However, the mechanism of action at the molecular level in human liver was not well characterized. In the present work human Chang Liver Cells (CLC) was used to study the gene expression pattern (Affymetrix human GeneChip U133 A) induced by 10 mM of Aflatoxin B1 for a period of 24 hrs. Differential gene expression between the normal CLC and Aflatoxin B1 treated CLC were found and analyzed. Nearly 400 genes were up regulated and 1000 genes were down regulated. For example, cyclin-dependent kinase inhibitor 1C, glutamine synthase, polymerase (DNA directed) gamma, N-acetylglucosaminyltransferase isoenzyme A and heterogeneous nuclear ribonucleoprotein D, were up regulated and are closely related to carcinogenesis. Conversely, genes, such as, SH3-domain GRB2-like endophilin B2, phosphoserine aminotransferase, N-acetyl galactosaminide alpha-2,6-sialyltransferase, G protein beta subunit-like, f-box and leucine-rich repeat protein 6 and villin 2 were down-regulated. These genes likely maintain tumor-specific characteristics and participate in key downstream regulatory mechanisms. This study may provide useful information on the carcinogenic mechanism of Aflatoxin B1.

Poster 2-22

OSTREOPSIS SP., A POSSIBLE ORIGIN OF PARROTFISH TOXIN

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The causative agent of parrotfish (*Scarus ovifrons*) poisoning is supposed to be palytoxin (PTX) or PTX-like substance (1, 2). However, its biogenetic origin is still unknown. Recently, a clone of toxic dinoflagellate *Ostreopsis* sp. adhered to the seaweed on which the parrotfish feeds was collected in August 1997 from the marine water of Tokushima Prefecture, the abundantly reported area of PTX poisoning in Japan, and cultured in ESM medium at our laboratory. After 16 days of rearing, about 4.0×10^5 cells were centrifuged for separating the cell pellet. The cell pellet was subsequently extracted with methanol by mild sonication, and centrifuged. The supernatant thus obtained was evaporated, defatted and partitioned between an aqueous layer and a 1-butanol. The crude 1-butanol layer showed lethal potency in mice test. The animals showed the symptoms of convulsion, drowsiness and collapse, and died within 48 h. Extract from the aqueous layer was found to be non-toxic. Haemolysis test was also performed as described previously (3, 4) with slight modification. The 1-butanol layer showed typical delayed haemolytic activity with mouse erythrocytes at sample concentrations of 10^2 cells/ml, 10^3 cells/ml and 10^4 cells/ml with the incubation time of 4 h. Further, the haemolytic activity with the above sample concentrations was found to drastically decline under the presence of anti-PTX antibody with the same incubation time. The haemolytic activity of 1-butanol layer toxin was also suppressed by ouabain, an inhibitor of PTX-induced haemolysis. *Ostreopsis* sp. toxin thus showed the characteristic behaviour of parrotfish poison PTX in this study. It is, therefore, hypothesized that *Ostreopsis* sp. might be the origin of PTX in parrotfish. However, further study along this line is underway.

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Poster 2-23

FORENSIC ANALYSIS OF A VICTIM OF PARALYTIC SHELLFISH POISONING MEDIATED BY THE XANTHID CRAB, *ZOSIMUS AENEUS*

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After ingestion of a specimen of the crab *Zosimus aeneus* (Xanthidae), an East Timorese adult male died within several hours. The crab contained a significant amount of paralytic shellfish toxins (PSTs) and these same toxins were identified in the gut contents, blood, liver and urine of the victim. Detection of PST-like bioactivity using the sodium channel and saxiphilin radioreceptor assays and confirmation of assay results with HPLC analysis allowed us to identify PSTs in the victim to further construct some of the toxicology of PSTs in humans.

Metabolism of the PSTs occurred with the ingested crab harbouring gonyautoxin 2, gonyautoxin 3 and saxitoxin (STX) whereas neoSTX, dcSTX and STX dominated the PSTs in the victim's urine. The PST composition in the gut contents, in both their identity and proportion, was intermediate between the eaten crab and the urine suggesting that toxin conversion commenced in the victim's gut. The lethal dose was calculated to be between 1-2 μg STX equivalents / kg based upon the concentration in the remains of the cooked crab. The victim's meal did not comprise solely of the toxic crab eaten and the possibility of other food items acting in a synergistic manner with the consumed PSTs cannot be discounted.

Poster 2-24

OCCURRENCE OF PARALYTIC SHELLFISH POISON IN THE STARFISH *ASTERINA PECTINIFERA* COLLECTED FROM KURE BAY, HIROSHIMA PREFECTURE, JAPAN

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Assays were made of paralytic toxicity of marine invertebrates inhabiting at the coasts of Hiroshima Bay, where the infestation of bivalves such as cultured oysters by paralytic shellfish poison (PSP) has been occurred. The starfish *Asterina pectinifera* collected at the estuary of Nikoh river, Hiroshima Bay, was found to contain moderate paralytic toxicity. Its highest toxicities as paralytic shellfish poison, (PSP) found on July 30, 1999 were 12.5MU/g for whole body, 11.0MU/g for integument tissues and 4.0 MU/g for viscera, respectively. The toxicity of integument was changed from 11.0MU/g to 3.9MU/g in one year. Its paralytic toxin principles were identified as PSP toxins, composing from protogonyautoxin 1 and 2(PX 1,2 or C1, C2), gonyautoxin 1 (GTX1), carbamoyl-N-hydroxy neosaxitoxin(hyneoSTX), decarbamoyl-STX (dcSTX) and, saxitoxin(STX), by HPLC and LC-MS. Among these PSP toxins, hyneo-STX was major accounting for 64.8 mole% of all. These PSP toxins contained in the starfish *A. pectinifera* considered to be transferred through food chain from bivalves or detritus living in the same area, which were contaminated with PSP.

Poster 2-25

PRELIMINARY RESULTS OF THE EVALUATION OF AN IMMUNOASSAY-BASED CIGUATOXIN TEST-KIT USING AUSTRALIAN FISH.

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Tropical and sub-tropical marine fish are often predisposed to contain ciguatoxins, which cause the serious illness in human consumers known as ciguatera fish poisoning. This illness is vastly under-reported, hence the true incidence in Australia remains unclear. Reports suggest numerous episodes occur each year along the eastern seaboard, many of which go unrecognised (or misdiagnosed). A previously trialed human diagnostic test is still unavailable, and further complicating the matter, the true prevalence of ciguatoxins in fish is largely unknown. A ciguatoxic fish is indistinguishable from a non-toxic fish, as the toxins are minute, colourless, odourless and tasteless. Most species that are reported to contain ciguatera are usually the direct result of a human intoxication episode. At present, consumers rely heavily on anecdotal evidence for guidance in which fish species may or may not cause ciguatera. Although testing procedures have previously been developed to detect the toxin in fish flesh, these are costly, labour-intensive and often non-specific. However, a simpler, rapid test-kit has been recently marketed overseas with the capability to detect nanogram quantities of ciguatoxins in fish flesh. Cigua-Check® is an immunoassay developed from ciguatoxin isolated from Northern Hemisphere fish species, and as such, there is some uncertainty as to its sensitivity to ciguatoxins in fish from the Southern Pacific and Indian Oceans.

This study involves evaluation of the applicability of the test-kit for the toxin profiles of Australian fish species. Numerous fish species found along the Queensland coast have thus far been tested, particularly those of commercial value. Initial evaluation of the sensitivity of the test-kit was performed on pure ciguatoxin isolated from Southern Hemisphere Moray eel. Subsequent testing included many fish implicated in ciguatera fish poisoning episodes or outbreaks along the east coast of Australia, as well as random negative fish controls. All fish that caused clinical symptoms in consumers gave positive results with the Cigua-Check® test-kit. However, confirmation is required using an established assay such as the brevetoxin competitive-binding assay. The results presented here are preliminary and at this stage cannot be used to validate the test-kit for commercial use on Australian fish species.

Poster 2-26

VENOM AND CNIDOME COMPARISONS BETWEEN 4 AUSTRALIAN CUBOZOANS.

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Four species of cubozoa found in Australian waters were selected for a venom and cnidome comparative study; these include two members of the Order Chirodropidae (*Chironex fleckeri* and *Chiropsalmus* sp.) and two from the Order Carybdeidae (*Carukia barnesi* and *Carybdea nr xaymacana*). Envenomations from two of these selected species are severe, *Chironex fleckeri* is responsible for numerous human fatalities, while *Carukia barnesi* envenomations are often life-threatening. Envenomations from the other two species are typically only mild in comparison. Analyses of their respective nematocyst types indicated that the two chirodropids appeared to contain the same complement of nematocysts, only differing in total number due principally to mastigophore sizes and percentages. In contrast, the two carybdeids exhibited quite distinct cnidomes, with *Carukia barnesi* having three different nematocyst types and *Carybdea nr xaymacana* having only two size classes of the same nematocyst type.

FPLC and SDS-PAGE analysis of the venoms indicated a higher degree of complexity in the cubozoans with the more severe envenomations. Both *Chironex fleckeri* and *Carukia barnesi* had more than twice the number of protein bands than their less venomous counterparts from the same Order. Chromatographic isolation of these proteins according to size also indicated stark differences in the prominent protein peaks. Of the chirodropids, *Chironex fleckeri* indicated a dominant protein of 16 kDa, while *Chiropsalmus* sp. showed a dominant protein of 12 kDa, and a secondary protein of 14 kDa. For the carybdeids, *Carukia barnesi* indicated a dominant protein of 12 kDa, and *Carybdea nr xaymacana* a dominant protein of 14 kDa with a secondary protein of 12 kDa.

We suggest the differences in venom toxicity are related to nematocyst types and feeding ecologies. The more venomous cubozoans tend to have a preference for vertebrate prey (fish), while the less-venomous cubozoans feed on invertebrates, predominantly shrimp. The large mastigophores in *Chironex fleckeri* and the euryteles in *Carukia barnesi* appear to be the primary penetrant nematocysts responsible for delivery of these more complex venoms.

Poster 2-27

MEASURING CARDIAC CHANGES IN BOX JELLYFISH ENVENOMED ANIMAL MODELS USING A VASCULAR DOPPLER

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Cardiac dysfunction is one of the major factors leading to clinical complications in humans. In previous studies, the cardio toxic effect of cubozoan venoms have been studied on numerous animal models, namely mammalian, and have used ECGs, blood pressure manometers and muscle contraction recorders. Little if any attempt has been made to measure cardiac responses in small invertebrate and vertebrate models that more closely represent the true prey, in both size and physiological similarity, which cubozoans feed upon. A new technique, using a portable and cost effective vascular doppler, a portable PC and sound analysis software allows for the rapid and continuous collection of both heart rate and contraction amplitude. This technique allows not only the time of death to be accurately determined, but also changes in cardiac parameters with time since envenomation. To date this technique has been successfully used on both invertebrate (freshwater crayfish) and vertebrate (fish) models for specimens as small as 5 grams in weight and can be used on animals that have either open or closed circulatory systems.

Poster 2-28

VISUALIZATION OF TETRODOTOXIN (TTX) IN THE SKIN OF TWO MARINE PUFFERS *TAKIFUGU VERMICULARIS* AND *CHELONODON PATOCA* UNDER LIGHT MICROSCOPE

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Micro distribution of tetrodotoxin (TTX) in the skin of two marine puffers *Takifugu vermicularis* and *Chelonodon patoca* was investigated by means of a monoclonal antibody (1) based immunoenzymatic technique under light microscope. The skin sections of *T. vermicularis* clearly showed different shapes of glands in the epidermis layer. TTX was localized as brown colour at the positively stained glands. Strong TTX antigen-antibody reaction was observed at cytoplasm of the glands. An opening extending from the gland towards super epithelial layer was visualized, through which TTX secretion is possibly performed. In the positively stained skin section of *C. patoca*, TTX was recognized in the succiform cells. The succiform cells were found in different stages of development. The erratic distribution of TTX was visualized in the super epithelial layer. Neither gland nor gland-like apparatus possessing TTX was apparent in the skin section of *C. patoca*. TTX antigen was not detected in the negative control sections. The present study, encompassing our previous reports (2, 3) reveals that micro distribution of TTX in skin varies in respect of species. Further study along this line on other puffer tissues is now in progress.

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Poster 2-29

STRUCTURE AND MEMBRANE INTERACTIONS OF EQUINATOXIN II, A β-SANDWICH THAT FORMS OLIGOMERIC PORES IN MEMBRANES

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Sea anemone cytolysins (actinoporins) are highly basic proteins of mass ~20 kDa that generate pores in membranes containing sphingomyelin (SM) by forming assemblies of 3-4 monomers in the membrane. The pores are permeable to small molecules and solutes and the resulting osmotic imbalance promotes cell lysis. The potency and properties of these cytolysins have prompted their evaluation as the toxic component of chimeric proteins targeted at tumour cells and human parasites. The actinoporins share no significant sequence identity with the bacterial pore-forming proteins such as α-hemolysin, where a heptameric complex forms the trans-membrane pore, making it likely that they have a unique structure and mechanism of action.

Equinatoxin II (EqtII) is a 179-residue cytolysin from the Mediterranean anemone *Actinia equina* L. (1). The structure of EqtII, determined by NMR on $^{13}C/^{15}N$-labelled protein, consists of two short helices packed against opposite faces of a β-sandwich structure formed by two five-stranded β-sheets (2). ^{15}N relaxation data show uniform backbone dynamics, implying that EqtII in solution is relatively rigid, except at the N-terminus. We have also investigated its interaction with micelles containing SM, an important constituent of membranes that are susceptible to lysis by this toxin. In addition, ^{2}H and ^{31}P solid-state NMR have been used to study the effect of EqT II on the structure and dynamics of bilayer lipids in multilamellar vesicles. The toxin appears to enhance slow motions in the membrane lipids and destabilize the membrane. This effect was greatly enhanced in SM-containing mixed lipid membranes compared with pure phosphatidylcholine bilayers.

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Poster 2-30

CONOPEPTIDES FROM THE VENOM OF THE MEDITERRANEAN WORM HUNTING *CONUS VENTRICOSUS:* BIOCHEMICAL, STRUCTURAL AND FUNCTIONAL CHARACTERISATION OF CONTRYPHAN-VN

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The venom of predatory marine snails of the gastropod genus *Conus* is a well-known rich source of neuro-active peptides representing useful tools as ion channels and receptors probes and as novel promising therapeutic agents. Conopeptides from fish-hunting cone snail venoms have been arising primary attention for their effects on mammals. The venoms of mollusc-hunting and especially worm-hunting species are less investigated. The biochemical characterization of the first conopeptide isolated from the venom of the Mediterranean worm-hunting *C. ventricosus,* Contryphan-Vn (1). as well as the several cDNA derived amino acidic sequences available in the data base, provided evidence that worm-hunting species contain an altogether wide and diverse array of conopeptides which may have novel specificity. Contryphan-Vn, a D-tryptophan containing nonapeptide shows a high sequence identity with previously characterised contryphans, all from fish–hunting and mollusc-hunting species. The presence of a charged residue -an intercysteine Lys- confers to the peptide a distinct surface electrostatic potential which suggested a distinctive molecular target. Although the target of all contryphans is still unknown, the occurrence in the three- dimensional structure of the dyad Lys –aromatic ring at a appropriate distance, shared by several potassium channel blockers (2) suggested a possible role of Contryphan-Vn as a potassium current modulator, with a minimal amino-acidic sequence. Preliminary experiments on vertebrate and invertebrate systems, such as rat fœtal chromaffin cells in primary culture, as well as dorsal unpaired median neurons (3) isolated from the cockroach nerve cord, showed diverse and interesting effects. Among others, in voltage-clamp conditions, Contryphan-Vn affected calcium sensitive and voltage-dependent potassium channels. Studies are in progress for uncovering the mechanism of the observed actions.

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Poster 2-31

THE MILKED VENOM FROM *CONUS GEOGRAPHUS* HOLDS MANY SURPRISES

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The milked venom derived from *Conus geographus*, a tropical carnivorous marine gastropod, is diverse in molecular constituents ranging from ~1000-8000 Da. Approximately 97% of these constituents contained disulfide bonds (1-7 disulfide bridges) as seen with corresponding molecular mass shifts after Tris(2-carboxyethyl)phosphine (TCEP) reduction, as observed by LC/MS and MALDI analysis. Many peptides were demonstrated to be post-translational variants of a number of parent constituents, these included the presence of 'O' linked carbohydrates, γ-carboxyglutamic acid (γ-Gla) and 4-*trans*-hydroxyproline (Hyp).

The comparison of the known molecular mass derived from whole duct venoms, duct venom sections and the previously published known conopeptides from this species [as reviewed by (1)], at present indicates little correlation to those masses observed within the milked venom.

However, two molecular masses were observed; these corresponding to Conantokin G and Conotoxin GS, the latter being correctly identified in both native and reduced environments. While Conantokin G demonstrated its inability to shift under TCEP reduction with its absence of disulfide bonds.

The illustrated contrast within duct, duct venom sections and milked venom constituents within *C. geographus* has become a common trend amongst other pisciovores members of the genus (e.g., *C. catus, C. magus, C. monachus, C. purpurascens C. striatus and C. tulipa*). These findings potentially highlight the biodiversity of the genus and/or that our understanding of conopeptide production and their utilization in prey immobilization is rudimentary.

Establishing this, we are provided with a novel approach to identify additional 'lead compounds' for pharmacological investigation.

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